REG. NO. 24918/64
ISSN: 0022-1155
CODEN: JFSTAB

JOURNAL OF FOOD SCIENCE & TECHNOLOGY

VOL. 39, NO. 1 JANUARY-FEBRUARY 2002

AFSTI

ASSOCIATION OF FOOD SCIENTISTS
& TECHNOLOGISTS (INDIA)

jfst

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

EDITOR-IN-CHIEF

Lokesh B.R.

Department of Lipid Science and Traditional Foods

Central Food Technological Research Institute, Mysore

EDITOR

Shamala T.R.

Central Food Technological Research Institute, Mysore

ASSOCIATE EDITORS

Krishna Kantha T.P., Mysore

Sakariah K.K., Mysore

Sreemathi Hariprasad, Mysore

Sri Hari B.R., Mysore

ADVISORS

Arya S.S., Mysore

Asopa V.N., Ahmedabad

Chauhan. G.S., Pantnagar

Dubash P.J., Mumbai

Kumar Bhatia, New Delhi

Manju Sharma, New Delhi

Pandit S.D., Mumbai

Patil S.F., Jalgaon

Prakash V., Mysore

Thakre N.K., Pune

Thyagarajan G., Chennai

PUBLICATION MANAGER

Daniel V.A., Mysore

EDITORIAL BOARD

INDIAN

Aravinda Prasad, Mysore
Bawa A.S., Mysore
Chincholkar S.B., Jalgaon
Gholap A.S., Mumbai
Guha Biswas N., New Delhi
Gupta M.N., Pantnagar
Gurumukh Singh, Pantnagar
Hirannaiah S., Mysore
Jamuna Prakash, Mysore
Jha Y.K., Pantnagar
Kochar G.K., Pantnagar
Krishnaprakash M.S., Mysore
Kumbhar B.K., Pantnagar
Kulkarni S.D., Bhopal
Lele S.S., Mumbai
Mahendrakar N.S., Mysore
Maheshwari P.N., Kanpur
Masoodi F.A., Hisar
Mital B.K., Pantnagar
Muktha Bai K., Mysore
Murali T.S.R., Secundrabad
Muralidhara, Mysore
Narpinder Singh, Amritsar
Paul Thomas, Mumbai
Pillaiyar P., Thanjavur
Potty V.H., Mysore
Rajesh Kapoor, New Delhi
Rajeshwar S. Matche, Mysore
Rama Murthy M.K., Bangalore
Ramesh M.N., Mysore
Sattigeri V.D., Mysore
Saxena S.K., New Delhi
Sharma G.K., Mysore
Sharma N., Izatnagar
Shekar Shetty, H., Mysore
Srikar L.N., Mangalore
Srinivas P., Mysore
Umaid Singh, Hisar
Upadhyay K.G., Anand
Varadaraj M.C., Mysore
Veerabasappa Gowda T., Mysore
Vijaya Rao D., Mysore

OVERSEAS

Brown C.M., Edinburgh, UK

Bulan Phithakpol, Bangkok, Thailand

Carlos Ricardo Soccol, Curtiba-PR, Brazil

Daniel Martinez-Carreara, Puebla, Mexico

Dietrich Knorr, Berlin, Germany

Eskine N.A.M., Canada

Gumbira-Said E., Bogor, Indonesia

Jacobsen M., Frederiksberg C., Denmark

Jan Bol, Zeist, The Netherlands

Jayatissa P.M., Colombo, Sri Lanka

Jayas D.S., Canada

Khalil I. Ereifej, Irbid, Jordan

Kwon T.W., Kyungsangnam-do, S. Korea

Lee Yuan Kun, Singapore, Rep. of Singapore

Leupold G., Freising-Weihenstephan, Germany

Lozano Y., Montpellier, France

Paolo Fantozzi, Perugia, Italy

Raimbault M., Montpellier, France

Ramaswamy H., Quebec, Canada

Rao M.A., New York, USA

Robin Y. -Y Chiou, Chiayi, Taiwan

Roiz C.A., Guatemala City, Guatemala

Roussos S., Reading, UK

Spencer J.F.T., Tucuman, Argentina

Viniegra-Gonzalez, Mexico City, Mexico

Zeki Berk, Haifa, Israel

Journal of Food Science and Technology accepts advertisements from manufacturers and dealers of chemicals, glassware, instruments, machinery as well as publishers/distributors of books/scientific journals. The Journal reaches research professionals, academicians, industrialists seeking information on the latest findings in all branches of science, technology and engineering of foods and food products. It has wide circulation at national and international levels.

The tariffs for advertisements are as follows :

Advertisement Rates (Per Insertion)

Inside Double Spread (4 Colours)	Rs	15,000	US $ 750
Back Cover (4 Colours)	Rs	12,000	US $ 600
Inside Front Cover (4 Colours)	Rs	10,000	US $ 500
Inside Back Cover (4 Colours)	Rs	9,000	US $ 450
Page Facing Inside Front Cover (4 Colours)	Rs	9,000	US $ 450
Inside Full Page (4 Colours)	Rs	8,000	US $ 400
Inside Half Page (4 Colours)	Rs	5,000	US $ 250
Inside Full Page (Black & White)	Rs	5,000	US $ 250
Inside Half Page (Black & White)	Rs	3,000	US $ 150
Inside 1/3 Page (Black & White)	Rs	2,000	US $ 100

Discount of 15% on regular tariff for advertisements booked for 2 to 3 insertions and 25% for 4 insertions and above are also offered.

Journal Size : Demy 1/4 (Trimmed Page Size 220 x 280 mm)

The advertisement matter may be in the form of art pulls, bromides, positives and blocks, which should be sent along with the relevant remittance in the form of DD favouring,

The Executive Secretary
AFST(I)
CFTRI Campus
Mysore - 570 013. India
☎ : 0821-515557, Fax : 0821-518670
e-mail : afsti@sancharnet.in

DR. S.C. BASAPPA

AFST(I) PRESIDENT FOR THE YEAR 2001-2002

Dr. Sadali Chickapaiah Basappa took his M.Sc(Agri) degree in 1962 and Ph.D. in Plant Pathology in 1970 from Madras University. After a short stint as Mycology Assistant at Agricultural College, Bangalore and Central Coffee Research Institute at Chickmagalur, he joined Central Food Technological Research Institute, Mysore in the year 1963. From humble beginnings, he rose to the position of Deputy Director and retired in the year 1996.

During his career, spanning over 33 years, he had made significant contributions in the areas of Mycotoxins, Yeast Biotechnology, Fermented Foods, Food Enzymes and Active Principles of Garlic by leading over 12 R&D projects. He was Head, Expediting Technical Co-ordination unit from 1992-95 and UGC visiting Professor and Resource Person (Microbiology from 1996-99) in Mysore University and Consultant in Food and Industrial Microbiology from 1999 to date.

He taught, trained and guided 10 M.Sc. students, 6 Ph.D. scholars and several candidates undergoing short term courses at CFTRI. He developed 10 processes, amongst which 3 processes viz. removal of aflatoxin from groundnut oil, production of Baker's yeast and process for the preparation of liquid banana have been taken over by entrepreneurs for commercial exploitation. He filed 2 CSIR patents on paddy liquor and catalase.

He was in Czechoslovakia as a Post-doctoral UNESCO and WHO Fellow at the Institute of Microbiology, Prague during 1969-70. He was a Visiting Scientist, under CSIR-CNRS Bilateral Exchange Programme in France in the year 1986. He visited England and attended conferences in London, Bath and Norwich.

He has published about 120 research and review (including the ones presented in symposia and conferences) papers in reputed journals both India and abroad. He edited a book on 'Yeast Biotechnology and wrote 2 documents on Aflatoxins for FAO/UNEP and currently in the process of completion of a monograph on Aflatoxin-Formation, Analysis and Control to be published by the Allied Publishers, New Delhi.

He has been a member of AFST(I) for over 25 years and has served for its growth in various capacities as Joint-Secretary (1982-83), Executive Secretary (1983-84), Vice-President (1992-93) and Trustee of AFST(I) Education and Publication Trust (1988 and 1998). He actively participated in organizing various symposia (ICFOST), International Conferences (IFCON) under the aegis of the Association during the above period. He is a member of Association of Microbiologists of India, Society of Biological Chemists of India, Mysore Science and Technology Forum, Indian Yeast Group, Association of Carbohydrate Chemists and Technologists of India.

He is the recipient of many awards, which include Laljee Godhoo Smarak Nidhi Award (1990), Dr. G.B. Manjrekar Memorial Award (1995), 20th Century Award for achievement (1998) by International Biography Centre, England and many more. He was conferred as Fellow of AFST(I) in the year 1991.

I am sure that our Association will grow from strength to strength of all spheres in its activities under his leadership. On behalf of the Editorial Board of *Journal of Food Science and Technology,* I wish him all success.

B.R. Lokesh
Editor-in-Chief

ANNOUNCEMENT

The last date for receiving the nominations for FELLOWS and other awards has been extended to 31, May 2002. This supersedes the announcement made on Page 20.

The date for receiving the applications for the scholarship has been extended to May 15th 2002.

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
RESOURCE CENTRE MANGALORE
5/4/02

INDEXED AND SELECTIVELY ABSTRACTED IN

- *Current Contents - Agriculture, Biology and Environmental Sciences*
- *Indian Food Industry*
- *NCI Current Contents*
- *Chemical Abstracts*
- *Biological Abstracts*
- *Food Science and Technology Abstracts*
- *Food Technology Abstracts*
- *Dairy Science Abstracts*
- *Nutrition Abstracts and Reviews - Series A - Human and Experimentals*
- *International Packaging Abstracts*
- *PITRA CD-ROM_Paper, Printing and Packaging Database*
- *Online PIRA Databases-Data-Star, Dialog, Orbit Search Service, PFDS Online and STN*
- *Fisheries Review*
- *Cambridge Scientific Abstracts - Microbiology, Biotechnology, Health and Safety Science*
- *Food Adlibra Dialog File 79.*
- *Food Adlibra Alerting Bulletin*
- *Food Adlibra Current Awareness Supplements for Food Science and Seafood*
- *Food Adlibra Current Awareness Supplements for Snacks and Confectionery*
- *Biology Digest*
- *NAPRALERT - Online Access via Bitel, Interest Compuserve, Prodigy and Phone Modem*
- *NAPRALERT - Off-line Access*
- *AGRIS Database of FAO*
- *The Engineering Index Monthly*

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 39 Number 1 January/February 2002

C O N T E N T S

REVIEW

Stickiness of Food Powders and Related Physico-chemical Properties of Food Components
S. Jaya, M. Sudhagar and *H. Das* 1

RESEARCH PAPERS

Oil Palm Fruit Peroxidase: Purification and Characterization
S.S. Deepa and *C. Arumughan* 8

Alkalinization Process for Cocoa (*Theobroma cacao* L.) Nibs and Quality Evaluation of the Powder by Response Surface Methodology
E.S. Bispo, V.L.P. Ferreira, H.C. Menezes, A.A. Vitali and *K. Yotsuyanagi* 14

Characterization of Cheese Whey and Its Suitability for Ethanol Production by Alginate Entrapped Yeast Cells
B.S. Sooch and *R.S. Singh* 21

Effect of Food Preservatives on *Acinetobacter* genospecies Isolated from Meat
S.C. Saha and *B.A. Chopade* 26

Effect of Primary Processing on Microbial Load of Cauliflower and Fenugreek
Saji Gomez, Lata and *Susanta K. Roy* 33

Mycotoxins, Mycotoxigenic Fungi and the Biochemical Changes in Makhana (*Euryale ferox* Salisb) Puffs of North Bihar
Basant K. Sinha, K.S. Ranjan and *T.N. Pandey* 38

***In Vitro* and *In Vivo* Availability of Iron from Bathua (*Chenopodium album*) and Spinach (*Spinacia oleracia*) Leaves**
Shashi Kala Yadav and *Salil Sehgal* 42

Studies on the Effect of Sterols on Ethanol Production
A.B. Chaudhari, S.K. Talegaonkar and *S.B. Chincholkar* 47

RESEARCH NOTES

Effect of Pre-harvest Chemical Treatments on Shelf Life of Pineapple Fruit
S. Talukdar and *F. Ahmed* 53

Journal of Food Science and Technology, devoted to original R & D contributions in all branches of Science, Technology and Engineering of Food Products, is a bimonthly publication of the Association of Food Scientists and Technologists (India), Mysore. Review Papers are also published depending on their merit.

No part of the journal should be reproduced without written permission of the Editor-in-Chief.

Publication of paper in the journal automatically transfers the copy rights from the authors to the journal.

The Editor-in-Chief reserves the privilege of editing the manuscript and adding or deleting relevant parts, to make it suitable for publication in the Journal.

The Editor-in-Chief assumes no responsibility for the statements and opinions expressed by the contributors.

Manuscripts for publication, books for reviewing and matter concerned with advertisements in the Journal should be addressed to the Editor-in-Chief, Journal of Food Science and Technology, AFST (I), CFTRI Campus, Mysore - 570 013, India.

The Instructions to Authors are published in the January-February issue of the journal. These are required to be followed *in toto*. No deviation is accepted.

New members of AFST (I) are entitled to receive 'Journal of Food Science and Technology' (JFST) or 'Indian Food Industry' (IFI).

Members opting for IFI can subscribe to 'Journal of Food Science and Technology' at a nominal rate.

Those members who were earlier getting or had opted for IFI, can now change the option (once in a year only), to get gratis copies of 'Journal of Food Science and Technology', if they so desire.

Advertisements from manufacturers and dealers of chemicals, glassware, instruments, machinery as well as publishers/distributors of books/periodicals are accepted.

Advertisements tariff rates are given on an earlier page in Box.

Correspondence regarding subscription and membership rates should be addressed to the Executive Secretary, AFST(I), CFTRI Campus, Mysore - 570 013. India.

All payments shall be made by Demand Draft or Money Order in favour of the Executive Secretary, AFST(I), CFTRI Campus, Mysore - 570 013. India.

In case of Indian Institutions and nationals, the subscription through agents is not entertained.

©AFST (I), CFTRI campus,

Mysore - 570 013 India

Biochemical Characterization of Aromatic and Non-aromatic Rice Cultivars
S. Basak, R.S. Tyagi and *K.N. Srivastava* 55

Microbiological Evaluation of Street Foods in Bhubaneswar
A. Das Mohapatra, C.C. Rath, S.K. Dash and *R.K. Mishra* 59

Microbial Quality of Some Turkish Honeys
Nur Ceyhan and *Aysel Ugur* 62

Packaging and Storage Studies on Biscuits Containing Finger Millet (Ragi) Flour
A. Selvaraj, N. Balasubrahmanyam and *P. Haridas Rao* 66

Effect of Processing and Cooking on Proximate Composition of Chickpea (*Cicer arietinum*) Varieties
M. Sood, S.R. Malhotra and *B.C. Sood* 69

Sensitivity of Catheptic Enzymes in Radurized Chicken Meat
S.N. Jamdar and *P. Harikumar* 72

Indian Soybean Varieties with Low Levels of Lipoxygenase Isozymes
Vineet Kumar, Sanjukta Patra, Anita Rani and *S. Prakash Tiwari* 74

Utilization of Peel in Plantain Wine Production
R. Ali-Simon and *N. Badrie* 76

Microbiological Status of Buffalo Meat from Slaughterhouses and Retail Outlets
Arun Kumar Tiwari, S.P. Singh and *Arun Kumar* 79

Studies on Osmotic-Air Drying Characteristics of Papaya Cubes
S. Kaleemullah, R. Kailappan and *N. Varadharaju* 82

D - Value of Trypsin Inhibitor in Soybeans in Tomato Sauce
R.N. Tripathi and *Nirankar Nath* 85

Optimization of Processing Conditions for Cottage Scale Production of Hurum
Charu Lata Mahanta and *Ritu Geu Goswami* 87

Effect of Pasteurization Methods on the Quality Characteristics of Yolk and Sponge Cakes
A. Bharti, P.C. Panda and *J. Sahoo* 91

Physico-chemical Changes During Ripening of Guava Fruit (*Psidium guajava* L.)
Ravi Agrawal, P. Parihar, B.L. Mandhyan and *D.K. Jain* 94

Sugarcane Juice Concentrate - Preparation, Preservation and Storage
Pushpa Singh, H.N. Shahi, Archna Suman and *P.C. Jain* 96

BOOK REVIEWS 99

PUBLICATIONS OF AFST(I)

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 1-7

Stickiness of Food Powders and Related Physico-chemical Properties of Food Components

S. JAYA, M. SUDHAGAR AND H. DAS***

Department of Agricultural and Food Engineering
Indian Institute of Technology, Kharagpur - 721302, India.

Stickiness is a property of sugar containing, hygroscopic amorphous powders. Stickiness and caking of food powders have been related to glass transition with the phenomenon governed by the difference of powder temperature and glass transition temperature of powder. Stickiness occurs at combinations of temperature and moisture content, corresponding to viscosity of amorphous material below a certain constant value. The dramatic decrease in viscosity above the glass transition temperature reduces the contact time between the particles and causes interparticle fusion resulting in stickiness. The sticky point temperature of different food powders is useful to predict storage condition of the powders. This review discusses the implications of stickiness of food powders related to glass transition temperature, viscosity, moisture content as well as the water activity.

Keywords : Stickiness, Glass transition temperature, Amorphous powder, Hygroscopy, Water activity, Caking and agglomeration.

Stickiness is a time-temperature dependent phenomenon often encountered during production and storage of dry powders. The term 'stickiness' refers to both particle-particle stickiness called Cohesion and particle-wall stickiness called Adhesion. Cohesion is an internal property of the powder and is a measure of the force holding the particles together. Particles upon coming into contact stick together as agglomerate, unless the bond between them is broken by applying force greater than the cohesive force. Adhesion is an interfacial property and is the measure of the force holding the powder to the surface of another material. Particles will stick to the surface, unless the bond between them and the structure is broken by applying force greater than the adhesive force.

Cohesion gives rise to the phenomenon of caking or lump formation in a powder bed, usually after exposure to high temperature and humidity. Dry hygroscopic foods such as fruit powder and instant coffee powder tend to form hard cake, due to the excessive temperature and uncontrolled moisture migration, when they are subjected to storage for a long period of time. The powder becomes no longer free flowing and will reconstitute less readily because the cake is less porous to moisture penetration and disintegration in water.

The dry products can be categorized into two major groups: non-sticky and sticky products. This categorization is strictly relative, since some non-sticky products will exhibit sticky behaviour under certain conditions. The non-sticky products can be dried using simpler drying methods. These products are relatively less hygroscopic and free flowing, e.g., skim milk powder, protein powder and gum powder. Sticky products are difficult to dry. During the drying process, they may either remain as syrup or stick to the dryer chamber wall. This can lead to low product yield and operating problems. Some of these products are so sensitive to the drying conditions that a few degree Celsius variations in temperature during drying turn them into a sticky and non-flowing mass (Bhandari et al. 1993). The fruit powders, which contain amorphous sugars, fall under the sticky category. The stickiness property of most fruit powders and mechanism of stickiness have been reported (Bhandari et al. 1997a,b; Masters 1985; Wallack and King 1988; Slade and Levine 1991; Roos and Karel 1991d)

**Corresponding Author, E Mail: hd@agfe.iitkgp.ernet.in **Present address - Department of Agricultural and Bio resource Engineering, University of Saskatchewan, Canada*

Mechanism of stickiness

The stickiness of powders depends on the physical characteristics of individual particles and the nature of the physical process by which the particles interact. The magnitude of these forces has been shown to vary over a wide range of values and the forces are transmitted in a variety of ways. The forces responsible for the stickiness have been classified as: i) Immobile liquid bridges; ii) Mobile liquid bridges; iii) Electrostatic force; iv) Solid bridges, and v) Mechanical interlocking (Papadakis and Babu 1992).

The force responsible for the stickiness of amorphous food materials is the immobile liquid bridges. Immobile liquid bridges are formed, when thin layers of viscous binders, adhering to the solid, are introduced between the particles. If an immobile adsorbed liquid film is present on the surface of the particles, the magnitude of the Van der Waals attractive force is increased due to decrease in effective inter particle distance. When the liquid bridge formation is increased, the adhesive forces at the solid liquid interface and the cohesive forces within the binder are utilized up to the point where the weaker one is overcome. Example of this type of liquid bridges is the caking of onion powder due to moisture absorption and is a case of humidity-based caking mechanism. In general, the water absorbed on particle surfaces forms a saturated solution with the soluble solids present in the particle and makes the particles sticky. Another type of immobile liquid bridge is formed between the nearly dry particles of spray-dried amorphous soluble materials at temperatures and/or humidity higher than what is called the powder sticky point. Examples of this type of materials are instant coffee powders, fruit juice powders and dried milk and whey powder. The mechanism of bridge formation is the viscous flow driven by surface energy (Downton et al. 1982; Wallack and King 1988). When two particles come in contact and if sufficient water can flow to build a bridge between them, the particles will stick together.

If sufficient water flow occurs, the particle surface viscosity will be decreased to a critical value of about 10^7 Pa.s (Downton et al. 1982).

Electrostatic forces occur between molecules, which bear a charge. The potential energy of interaction between the molecules is inversely proportional to the distance between the molecules. This electrostatic force is the potential contributor to the adhesion phenomena. However, electrostatic forces cannot explain all types of adhesion. It, however, plays a role in the formation of ionic bonds and ionic crystals, e.g., sodium chloride (Alphonscs 1997).

There are three forms of mobile liquid bridges that occur with amorphous powders, viz. a) Pendular state b) Capillary state and c) Fenicular state. In the Pendular state, wet agglomerates are formed by forces resulting from the presence of mobile liquid between the particles. The void space between the particles is partly filled with the liquid, which forms bridges between the individual particles. In the Capillary state, the void space between the particles is completely filled with liquid, which extends approximately to the edge of the pores. In transition state, gas is present inside the granule, but the liquid is still present as a continuous network. This condition is called Fenicular state. Since the mobile liquid bridges are present as a continuous network, they interact with all other molecules present in the system and also increase intermolecular forces due to decrease in intermolecular distance (Papadakis and Babu 1992.

Solid bridges are formed by sintering, chemical reactions, melting, hardening, bonding agents and crystallization of dissolved materials. Mechanical interlocking can occur in particles that are needle-shaped crystals. The lumps formed are generally very strong and can be broken only by breaking the particles. Interlocking can also occur between the powder and a rough surface. The reason for the strong bond of this type is purely a matter of greater physical area of contact. Compared to plane surfaces, the surfaces of needle-shaped crystals or rough surfaces have more area of contact and have larger surface interaction.

Measurement of sticky point temperature

Sticky point temperature is defined as the temperature at which caking is instantaneous with slow stirring. A sticky-point apparatus developed by Lazar et al. (1956) has been in use since then by several others (Downton et al. 1982; Wallack and King 1988; Buhler and Liedy 1989). The test assesses the influence of temperature and moisture content on powder cohesion and is mainly applicable to nearly dry powders, which form immobile liquid bridges of water present in the particles.

The test apparatus developed by the present authors is shown in Fig.1. A powder sample (4) having known moisture content is placed in the sample holder (1). The powder is isolated from atmosphere via a mercury seat (2). The test tube is immersed in a constant temperature water bath. The bath temperature is slowly raised, while the powder is intermittently stirred by hand through a small stirrer (3) which is rigidly fixed

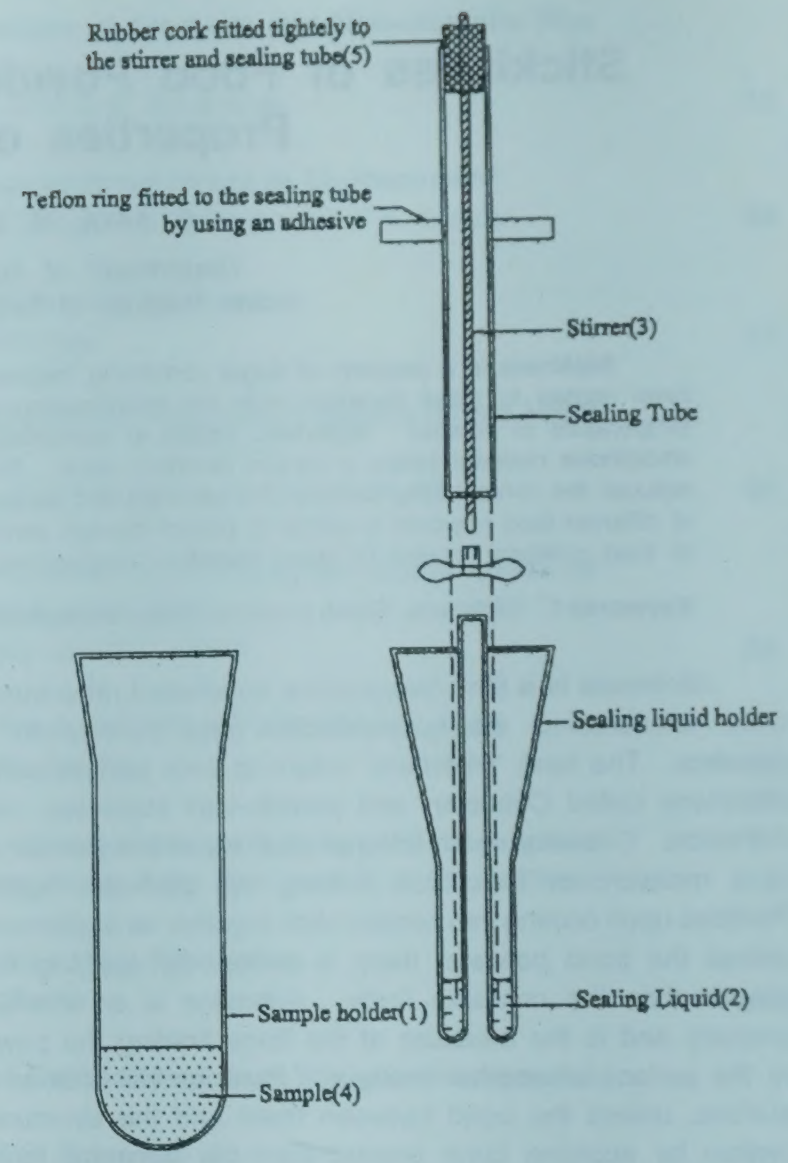
Rubber cork fitted tightely to the stirrer and sealing tube(5)
Teflon ring fitted to the sealing tube by using an adhesive
Stirrer(3)
Sealing Tube
Sealing liquid holder
Sealing Liquid(2)
Sample holder(1)
Sample(4)

Fig. 1. Exploded view of sticky point apparatus

to the rotating body (5) embedded in the sample. At a certain temperature, the force required to turn the propeller is increased dramatically. The temperature at which this occurs is usually distinct and is referred to as the sticky point temperature of the powder. The test is repeated at other moisture contents of the powder and the results are plotted as sticky-point temperature versus product moisture content. Typical sticky point curves for different food powders as obtained from the available data (Downton et al., 1982; Lazar et al., 1956; Wallack and King, 1988) by using this method are shown in Fig. 2, which shows that with the increase in moisture content, the sticky point temperatures of the powders reduce.

Papadakis and Babu (1992) and Bhandari et al. (1993) measured the sticky point temperature by visual observation of adhesion of powder on a polished metal surface, which was heated, and its temperature regulated over its length. The sticky point temperature was taken as the lowest temperature at which the powder was found to adhere to the metal surface.

Chuy and Labuza (1994) measured the sticky point temperature of powders contained in sealed glass tube and fitted with a propeller. They used 5 g powder humidified to different water activities and placed inside the glass tube. The tube was immersed into an oil bath and heated at 0.33 to $50^0 Cmin^{-1}$. The tube was lightly tapped and the propeller was

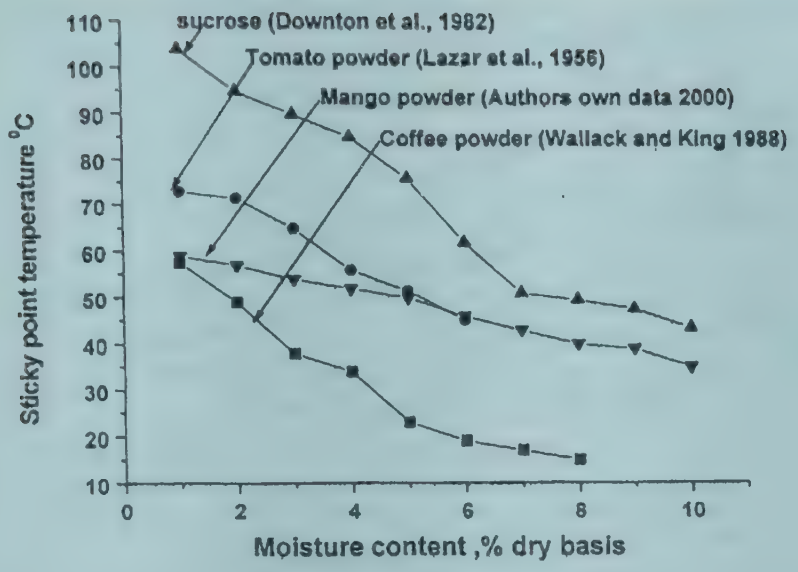

Fig. 2. Sticky point temperatures of tomato, coffee and mango powders and sucrose at different moisture contents

twisted by hand at every 5^0C increase in temperature. The temperature inside the sealed tube and the temperature of the bath were measured simultaneously. The sticky point temperature was recorded as the temperature of the powder at which the force required to turn the propeller increased sharply.

Physico-chemical properties of amorphous food polymers

An amorphous food is formed under non-equilibrium conditions either by removing the dispersing medium (such as water) or by rapid supercooling (e.g., quick freezing). This material is not at thermodynamic equilibrium and therefore is unstable. A dry food normally consists of amorphous substances such as simple sugars and polysaccharides. These materials are metastable and exhibit low phase transition temperature. They are very sensitive to change in moisture content and exhibit stickiness, caking and agglomeration. Basically, the sticky behaviour depends on the temperature and the amount of sugars and moisture present in these materials.

TABLE 1. GLASS TRANSITION TEMPERATURES OF ANHYDROUS SUGARS AND CARBOHYDRATE POLYMERS AND THEIR PHYSICAL PROPERTIES AND STICKINESS BEHAVIOUR (Bhandari et al. 1997a and Bhandari and Howes, 1999*)

Food material	Molecular weight	T_g ^{0}C	Relative hygroscopicity	Melting point, ^{0}C	Solubility in water at 60^0C (kg.kg solution^{-1})	Relative stickiness
Fructose	180	5	Very high	105	89	Very high
Glucose	180	31	High	146	42	High
Sucrose	342	62	Moderate	186	71	Moderate
Maltose	342	87	Fair	165	52	Fair
Lactose	342	101	Low	223	35	Low
Maltodextrin						
DE 36**	500	100				
DE 25	720	121				
DE 20	900	141				
DE 10	1800	160				
DE 5	3600	188				
Starch		243 (predicted)				

* Reproduced from the original papers by Courtesy of Marcel and Dekker, Inc, USA and Elsevier Science, UK.

** DE : Dextrose equivalent

Glass transition temperature

Glass transition temperature (T_g) is the temperature at which an adhesive substance looses its flexibility and becomes hard, inflexible and 'glass like'. At glass transition temperature, amorphous food polymers are transformed into a viscous-liquid or rubbery state from the solid amorphous state. A dramatic change in molecular mobility and physical properties takes place at the Glass transition temperature (Slade and Levine 1991).

Glass transition temperature (T_g) is the temperature at the onset or midpoint of the change in heat capacity (Roos 1995) of the material and is measured by using Differential Scanning Calorimeter. For food materials, the value of T_g vary between -135^{0}C for pure water and above 250^{0}C for a high molecular weight food polymer, such as starch (Roos 1995). In frozen foods, glass transition occurs over a temperature range that is dependent on food composition and the amount of unfrozen water (Slade and Levine 1991; Roos 1995) present in the material. There are various relaxations associated with the glass transition depending on the extent of molecular arrangements during glass formation and the physical aging in the glass state (Roos 1995).

Some of the important physical properties of sugars and carbohydrate polymers that could be responsible for the stickiness are molecular weight, glass transition temperature T_g, melting point and solubility. Table 1 shows that the stickiness increases with the increase in the solubility and reduction in melting point. In the case of fructose, it has been observed that it never attains a perfect solid state during normal drying condition even though it can be dried to a very low moisture level. It exists in a very viscous and deformable plastic state.

Slade and Levine (1991) and Roos and Karel (1991d) reported that sticky point temperature of food powders decreases with decreased molecular weight and T_g value. Levine and Slade (1986) and Roos and Karel (1991c,d) reported that materials such as fruit juice powders with high amount of monosaccharides e.g. fructose exhibit low T_g values and thereby low sticky point temperature. Stickiness of such products can be reduced and the stability increased by adding compounds having higher T_g values. Glass transition temperatures along with other properties of anhydrous sugars and carbohydrate polymers are shown in Table 1. Since the T_g value increases with increasing molecular weight, the high molecular weight amorphous material like maltodextrin is generally used to increase the glass transition temperature, decrease stickiness and improve product stability.

Bhandari et al (1993) produced black currant, raspberry and apricot juice powders by drying a mixture of the juice and

maltodextrin having 6 Dextrose Equivalent (DE). They fixed the respective proportion of juice solid and maltodextrine in the ratios of 65:35, 55:45 and 60:40, respectively. They observed that the stickiness varied with the composition of the juice and decreased when the proportion of the juice solids was reduced. Lazar et al (1956) and Brennan et al. (1971) observed stickiness of tomato powders and orange juice concentrate during processing. These studies showed that stickiness was promoted by increased relative humidity and temperature of atmosphere.

Viscosity of amorphous powders

Downton et al (1982) proposed a mechanistic model, which could describe quantitatively, the tendencies for particles to stick to one another or to a wall. If two dry particles come in contact, they may or may not stick, depending upon whether sufficient liquid flow can occur to build a bridge between the particles that is strong enough to resist subsequent mechanical deformations. The driving force for the flow of liquid during the contact is surface tension. Resistance to liquid flow is measuring the viscosity of the powder material.

The viscosity of amorphous materials has been found to decrease at temperature 10 to 20^{0}C above the T_g value (Roos and Karel 1991a). At this temperature and moisture content, a dramatic decrease in viscosity below about 10^7 Pa.s is observed, which correlates with the end point of the glass transition. The decrease in viscosity above the glass transition temperature range causes inter particle 'fusion, resulting in stickiness, caking and structural collapse due to viscous flow, as the material cannot support its own weight (Roos 1995). Structural collapse is the phenomenon that causes loss of quality of dehydrated foods. The viscosity at low water content can be considered to be constant, since the amount of water present is insufficient to decrease the value of T_g to a value below the ambient temperature.

Viscosities at which stickiness occurred (Fig. 3) in sucrose powder were measured by Downton et al. (1982), who used the well-known falling ball method for finding the viscosity values lower than 10^7 Pa.s and Weissenberg rheogoniometer for the measurement of higher values of viscosities. From

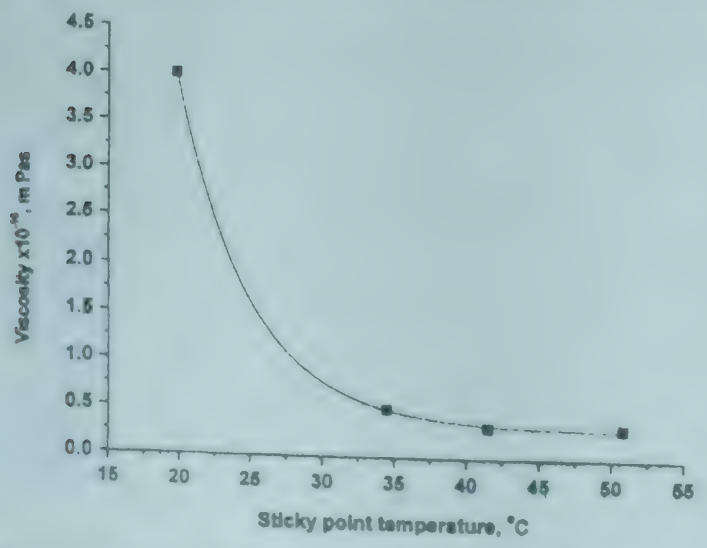

Fig. 3. Sticky point temperature and viscosity of the amorphous sucrose (Drawn from the data of Downton et al., 1982)

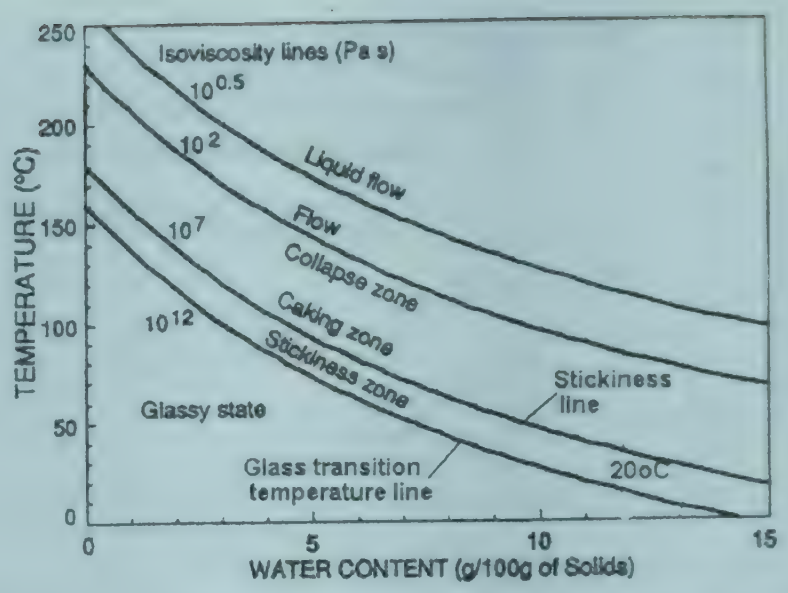

Fig. 4. Iso-viscosity lines of maltodextrin (DE 10) at various water content and temperature (Reprinted from Roos 1998, page 78 by Courtesy of Marcel and Dekker, Inc.)

Fig. 3, it may be observed that the viscosity of sucrose powder lies within a limited range of 0.32×10^7 to 4.0×10^7 Pa.s.

Downton et al. (1982) also reported that amorphous solids at very low temperature have viscosity greater than 10^{12} Pa.s. As the temperature rises, the viscosity decreases to a critical value of around 10^7 Pa.s, where they first become sticky. The temperature associated with this critical viscosity decreases with increasing water content.

Effect of water content on changes of viscosity of maltodextrin (DE10) above the Glass transition is shown in Fig. 4 (Roos and Karel 1991b). The Figure shows isoviscosity lines and the critical viscosity for stickiness and other changes like caking, collapse and finally the liquid like flow of maltodextrin in relation with temperature and water content. The glassy state of the maltodextrin has a viscosity of 10^{12} Pa.s (Fig. 4) and the T_g value of anhydrous maltodextrin (DE10) is 160^{0}C. As the temperature is increased to about 180^{0}C, it looses its glassy character and attain the sticky nature. From Fig. 4, it is also observed that the glass transition temperature line and stickiness line are almost parallel and are separated by a temperature difference of about 20^{0}C. This indicates that the stickiness of the product at given moisture content will occur at about 20^{0}C above the glass transition temperature. The Figure also shows that at low water content and temperature greater than the critical, the viscosity will be lowered. It is, therefore, important to maintain both the water content and the temperature below the critical value to maintain glassy and amorphous state of materials.

The viscosity η (mPa.s) of a powder at a temperature T (^{0}C) can be predicted using WLF equation (Roos and Karel 1991e).

$$\log \frac{\eta}{\eta_s} = \frac{-17.44\ (T - T_g)}{51.6 + (T - T_g)} \quad \text{..... (1)}$$

where, T_g(^{0}C) = glass transition temperature of the product and η_s = the viscosity at T_g.

Water activity and moisture content of amorphous food polymers

Rates of physico-chemical changes in foods often depend on water content and water activity. Mobilization of molecules occurs above a certain water content and water activity, which is close to the monolayer moisture content of the material. The amount of water that is lower than the monolayer value is 'nonsolvent' and above the monolayer, the water is assumed to be 'solvent' for the reactants. However, in amorphous foods at low moisture content, the reactants get "frozen" into solid during the removal of water and the molecules may be mobile depending on the temperature and the extent of 'plasticization'. Mobilization of molecules occurs most probably as the temperature is increased to above T_g value or the water content is adequate to depress the T_g to below the ambient temperature. Such mobilization affects reaction rates as a result of increased diffusion of molecules above the T_g value. Since amorphous foods are hydrophilic, they are also very hygroscopic in nature. They result in reducing the T_g value and the viscosity.

Roos (1998) stated that the most common caking mechanism in sugar-rich food powders is 'plasticization' due to water sorption and subsequent inter-particle fusion. In general, food materials at higher temperatures show an increase in water activity at constant moisture content. In these foods, as the temperature is increased, the hydrogen bond between water molecule and the OH group of the food solid breaks and the water becomes free. This increases the water activity. Even though there is no moisture gain or loss, this increase in water activity further disturbs the hydrogen bond between the water molecule and the OH group.

However, in foods containing sugars, an opposite trend is observed. In these foods, at higher temperatures, dissolution of sugars takes place in the newly formed free water. This results in decrease in water activity with the increase in temperature. Fig. 5 shows the relationship between the water activity and moisture content for fructose at 80°C and 30°C and the sucrose at 80°C and 35°C (Iglesias 1982). It may

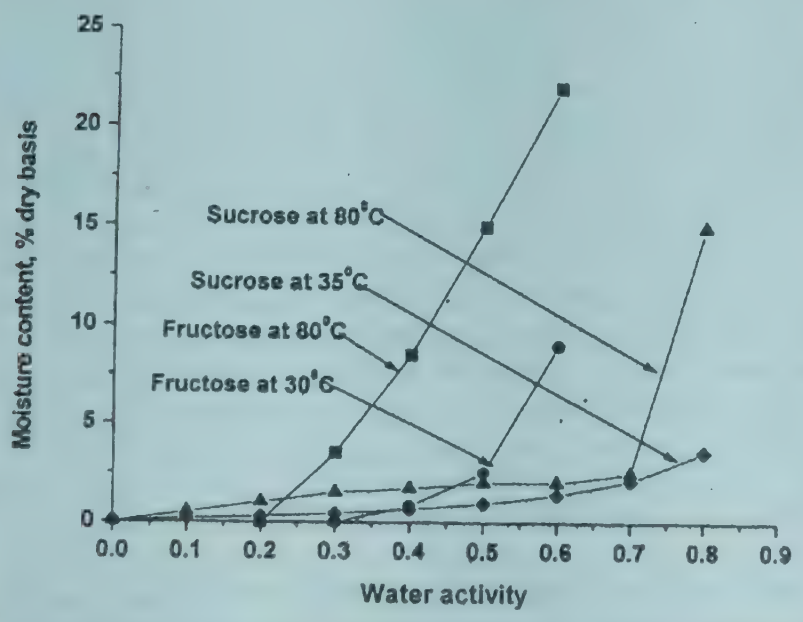

Fig. 5. Relationship between water activity and moisture content for various food components at various temperatures (Drawn from the data of Iglesias, 1982)

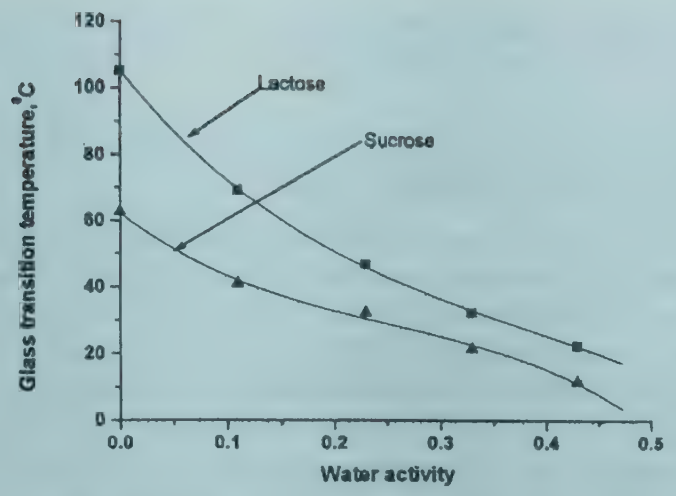

Fig. 6. Glass transition temperature of lactose and sucrose as a function of water activity (Drawn from the data of Roos 1991b)

be observed from the Figure that the moisture content of the two sugars at a particular water activity is less at lower temperature than at higher temperature. The Figure also shows that in comparison to sucrose, fructose exhibits lower water activity at higher moisture content. This is due to the higher solubility of fructose (Table 1) than sucrose at a given temperature. The low T_g value also increases the stickiness in foods containing more fructose than sucrose.

Roos (1987) observed the relationship between water activity and T_g value for several amorphous foods. Fig. 6 shows such a relation for lactose and sucrose (Roos 1995). From this type of relationship, one can predict the effective water activity level for storage in relation with glass transition temperature to avoid stickiness and caking of food powders. The relationship between T_g and water activity at constant temperatures provides a simple method of prediction of the effect of relative humidity, which causes the stickiness and finally caking during storage. The sticky point temperature is the endpoint temperature of the glass transition. Such prediction is useful in evaluating storage stability of various low moisture cereals and snack foods. Fig. 7 shows the

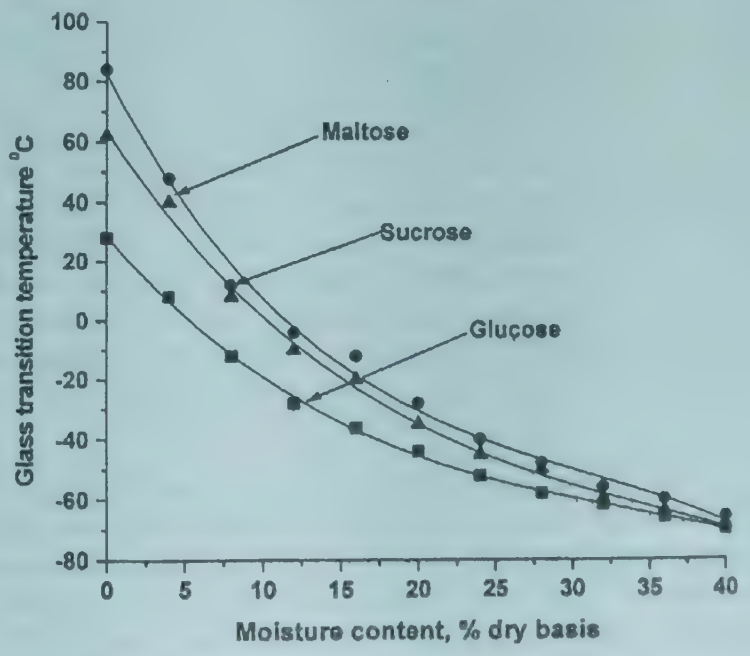

Fig. 7. Effect of water content on T_g of amorphous food materials (Drawn from the data of Roos and Karel 1991e)

relationship between Glass transition temperature and water content of glucose, maltose and sucrose (Roos and Kavel 1991c)

Shelf life prediction of powder using sticky point temperature and moisture content

A powdery material is normally stored in flexible packages. Sealability, low moisture permeability, opaqueness and printability are the qualities needed for the packaging materials. Since a single material cannot meet the above qualities, several materials arranged in layers are often used. Even with the adoption of the best possible technology, the moisture permeability in these materials cannot be totally eliminated.

Moisture content of a powder stored inside a package will therefore increase due to migration of moisture from atmosphere. Stickiness between the individual particles of powder will result in caking and agglomeration. As the stickiness is a function of moisture content and temperature (Fig. 2 and 4), the sticky point moisture content at particular storage temperature can be termed as the critical moisture content, X_c (kg water.kg dry solid^{-1}) of the powder.

When a powdery material is contained inside a flexible package and stored in an atmosphere maintained at a temperature, T (^{0}C) and relative humidity Rh, (fraction), the moisture content X (kg water.kg dry solid^{-1}) and the corresponding water activity, a_w of the powder will continue to change with time. The time t (days) required for the moisture content of the powder to increase from an initial value of X_i (kg water.kg dry solid^{-1}) to its critical value X_c (kg water. kg dry solid^{-1}) can be expressed as

$$t = \frac{W_s}{PKA} \int_{X_i}^{X_c} \frac{dX}{Rh - a_w} \quad \text{..... (2)}$$

where, W_s (kg) = the dry weight of the powder, P (Pa) = the saturation vapor pressure of water at T (^{0}C); K (kg water. m^{-2}. day^{-1}.Pa^{-1}) = the permeability of the packaging material; A (m^2) = surface area of the packaging material; a_3 (fraction) = the water activity of the powder at T (^{0}C) and X (kg water.kg dry solid^{-1}) = the moisture content of the powder.

The initial value of the moisture content, X_i should normally be the monolayer value of the powder and it can be estimated from the moisture sorption data available at the temperature of storage by using the GAB or BET equation. As moisture migrates into the packaging material, the value of a_w will rise and that of (Rh-a_w) will reduce. Since the a_w and X relationship in non-linear (e.g. BET, GAB, equations are nonlinear) the value of the integral of Eqn. (2) has to be estimated numerically.

Eqn (2) was used by the authors to determine the shelf life of mango powder. When the moisture sorption data of mango powder were fitted to the GAB equation, the value of monolayer moisture content obtained was .0.0164 kg water.kg dry solid^{-1} and the corresponding water activity value was 0.095. The value of X_i in eqn (2) was therefore taken as 0.0164.

The authors took 0.005 kg (i.e., W_s = 0.00492) mango powder in a 75 m thick LDPE package having 7.68x10^{-11} kg water.m^{-2}.day^{-1}.Pa^{-1} permeability and stored at 38^{0}C, 90% relative humidity (i.e. Rh = 0.9). The surface area of the packaging material was 0.0256 m^2. The a_w - X relationship of the powder at 38^{0}C was expressed by the equation : $a_w = 0.6001 - 13.425*X + 195.77*X^2 - 775.48*X^3$, which was used for the numerical integration of Eqn. (2). From Fig. 2, it can be seen that the sticky point moisture content of mango powder at 38^{0}C is 0.094 kg water.kg dry solid^{-1} i.e. X_c = 0.094. With these data, when put in Eqn. (2), the shelf life of mango powder was estimated as 54.04 days.

To check the validity of the above model, the authors observed that the powder required 20 days to increase its moisture content from 0.0711 kg water.kg dry solid^{-1} to 0.0964 kg water.kg dry solid^{-1}. When calculations were made by putting X_i = 0.0711 and X_c = 0.0964 into Eqn (2), the value of t obtained was 18.78 days. Thus, the actual shelf life was 1.065 (i.e., 20/18.78) times the theoretically predicted shelf life. It was, therefore, concluded that the shelf life of the mango powder can be predicted by using the above model.

Problems of drying sticky and amorphous food materials

The "collapse" of the dehydrated food during freeze drying, stickiness of the product to the walls of the spray dryer during drying; caking and agglomeration of powder during storage are some of the problems associated with amorphous foods that are related to the Glass transition temperature.

During drying, the sugar-rich products may either remain as syrup or stick on the dryer wall. There is also the problem of unwanted agglomeration during the conveying of powder. These lead to low product yield and operating problems (Bhandari et al. 1997a).

The first step of spray drying is the atomization of feed. Normally, a concentrated solution is atomized into fine droplets of 20-150 mm diameter (Masters 1985). As water evaporates, the solutes in the droplets get concentrated. The physical state of the product changes from liquid solution to syrup and finally the solid. If the viscosity of the product remains below a critical value of 10^7 Pa.s, the product may remain as syrup even at low moisture levels (Downton et al. 1982). Depending on the product characteristics, composition and drying conditions, the surface of the dried droplets may remain plastic, resulting in stickiness on the drier wall or another droplet.

Hot air drying (either fixed or fluidized bed) is normally employed to dry solid foods. During drying, sugar-rich products such as fruit leathers may appear wet even at very low moisture content. Excessive disruption of skins or tissues in fruits results in leaching of sugar on the surface and poses problems in drying (Bhandari and Howes 1999).

Control of powder stickiness during drying

Introduction of cold air : Introduction of cold and dehumidified air towards the end of the drying process especially at the lower end of the drying chamber can result in formation of solid particle surface and reduce stickiness of

the powder to the dryer wall (Lazar et al. 1956).

Partial crystallization of sugar : It reduces hygroscopicity of powder. This is normally practised in producing whey and lactose powders. An extended fluidized bed integrated with two stage drying can also be used to crystallize a part of sugar. This does not solve the stickiness problem but could produce partially crystalline, solids having low hygroscopicity that will overcome caking during storage (Masters 1985)

Hynd (1980) reported that in dry whey powders, amorphous lactose is responsible for the stickiness, which can be reduced by crystallizing the amorphous lactose to α-lactose monohydrate. Thus, a separate lactose crystallization process is carried out first and then the crystals along with the mother liquor are spray dried.

Cooling of the dryer chamber wall : A cool chamber wall will solidify the outer surface of the thermoplastic particles and minimize the particles sticking to the wall. This method was found to improve the process but did not solve the problem. The reason is that the cold chamber wall will also cool the surrounding environment and cause an increase in the relative humidity of the air close to the wall surface (Brennan et al. 1971)

Scrapping of chamber wall : An intermediate sweeping of chamber wall with dehumidified cold air can remove loosely sticked particles from the wall. This method can be useful for relatively less thermoplastic sugars such as lactose and sucrose.

Addition of drying aids : Addition of drying aids such as maltodextrin to the feed increases the T_g value and the stickiness problem is considerably reduced (Bhandari et al. 1993; Masters 1985).

Spray dryer chamber design : Masters (1985) reported that for sticky products, special spray dryer chamber designs have been developed. These include : a) Double-wall-drying chambers, whereby cooling air is used to maintain the dryer wall surface temperatures below about 50^0C. This limits the wall deposits and prevents product degradation during hold-up at the wall (e.g. tomato paste drying). For very sticky products flat chambers are employed. b) Chambers with wall-sweeper slots from where, secondary-cooling air is introduced into the chamber tangential to the primary air. and c) Chambers with air bottom, which rotates slowly close to the wall are also used for drying purposes. The air broom system arm contains a row of nozzles that directs compressed air onto the wall surface. The air broom system of spray drying was used by the authors for production of banana powder in an industrial dryer.

References

Alphonses VP (1997) Adhesion and Adhesives Technology an Introduction. Honser Publication, Munich Vienn, New York, pp 69-72, 118-130

Bhandari BR, Senoussi A, Dumoulin ED, Lebert A (1993) Spray drying of concentrated fruit juices. Drying Technol 11(5) : 33-41

Bhandrai BR, Datta N, Howes T (1997a) Problem associated with spray drying of sugar-rich foods. Drying Technol 15(2) : 671-684

Bhandari BR, Datta N, Crooks R, Howes T, Rigby S (1997b) A semi-empirical approach to optimize the quantity of drying aid required to spray dry sugar-rich foods. Drying Technol 15(1) : 2509-2525

Bhandari BR, Howes T (1999) Implication of glass transition for the drying and stability of dried foods. J Food Engng 40 : 71-79

Brennan JG, Senoussi A., Jowitt R (1971) A study of some of the factors affecting the spray drying of concentrated orange juice on a laboratory scale. J Food Technol 6 : 295-307

Buhler W, Liedy W (1989) Characterization of product qualities and its application in drying process development Chem Eng Process 26(1) : 27-34

Chuy LE, Labuza TP (1994) Caking and stickiness of dairy based food powders as related to glass transition. J Food Engng 56(1) : 43-46

Downton GE, Flores-Luna JL, King CJ (1982) Mechanism of stickiness in hygroscopic amorphous powders, Industrial Engineering Chemistry Fundamentals 21 : 447-451

Hynd J (1980) Drying of whey J Society Dairy Technol 33(2) : 52-54

Iglesias, Hector A. (1982) Handbook of Isotherms. Academic Press, New York, pp 99, 223, 224

Lazar ME, Brown A.H, Smith GS, Wang FF, Lindquist FE (1956) Experimental production of tomato powder by spray drying. Food Technol (10) : 129-134

Levine H, Slade L (1986) A food polymer science approach to the practice of cryostabilization technology. Comm Agric Food Chem 1:315-396

Masters K (1985) Spray Drying Handbook, 4th edn. Longman Scientific and Technical, England

Papadakis SE, Babu RE (1992) The sticky issues of drying. Drying Technol 10(40) : 817-837

Roos YH (1987) Effect of moisture on the thermal behaviour of strawberries studied using differential scanning calorimetry. J Food Sci 52 : 146-149

Roos YH (1995) Glass transition in low moisture and frozen foods : effects on shelf life and quality. Food Technol 49(10) : 97-102

Roos YH (1998) Role of water in phase-transition phenomena in foods. In : Phase / State Transitions in Foods - Chemical, Structural and Rheological Changes. Rao MA, Hartel RW (eds) Marcel Dekker Inc., New York, pp 57-86

Roos YH, Karel M (1991a) Phase transitions of mixtures of amorphous polysaccharides and sugars, Biotechnol Progress 7 : 49-53

Roos YH, Karel M (1991b) Plasticizing effect of water on thermal behaviour and crystallization of amorphous food models. J Food Sci 56 : 38-43

Roos YH, Karel M (1991c) Phase transition of amorphous sucrose and sucrose solution. J Food Sci 56 : 38-49

Roos YH, Karel M (1991d) Amorphous state and delayed ice formation in sucrose solutions. J Food Sci 56 : 1676-1681

Roos YH, Karel M (1991e) Water and molecular weight effects on glass transition in amorphous carbohydrates and carbohydrate solution. J Food Sci 56 : 1676-1681

Slade L, Levine H (1991) Beyond water activity, Recent advances based on an alternative approach to the assessment of food quality and safety. Crit Rev Food Sci. Nutr. 30 : 115-360

Wallack DA, King CJ (1988) Sticking and agglomeration of hygroscopic, amorphous carbohydrate and food powders. Biotechnol Progress 4(1) : 31-35

Received 27 November 2000; revised 9 July 2001; accepted 5 December 2001

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 8-13

Oil Palm Fruit Peroxidase : Purification and Characterization

S. S. DEEPA AND C. ARUMUGHAN*

Agro-processing Division, Regional Research Laboratory,
Thiruvananthapuram - 695 019, India

Soluble peroxidase from oil palm fruit was purified by $(NH_4)_2SO_4$ precipitation, molecular exclusion chromatography and anion exchange chromatography. The fold purification obtained was 152 with an yield of 16%. Gel filtration on Sephadex G-200 indicated a molecular weight of 200 kDa, while the sub-unit molecular weight was 48 ± 2 kDa by SDS-PAGE. It had an optimum pH of 5 and exhibited very high pH and thermal stabilities. K_m for guaiacol, ABTS and pyrogallol were 2 mM, 1.6 mM and 1.2 mM, respectively. The enzyme exhibited indole-3-acetic acid oxidase activity and needed H_2O_2 and 2, 4-Dichlorophenol for the oxidase activity.

Keywords : *Elaeis guineensis*; Oil palm fruit; Enzyme purification; Peroxidase; Indole-3-acetic acid oxidase; Lipid oxidation.

Lipid oxidation is one of the major causes of food spoilage, as it leads to development of off-flavours and odours in edible oils and fat containing foods. (Nawâr 1985). The major causes for the oxidative deterioration of food lipids are autooxidation by free radical chain reaction, photo-oxidation (Frankel 1980; Nawar 1985) and enzymatic oxidation catalyzed by lipoxygenase (Galliard and Chan 1980; Hildebrand 1989; Siedow 1991). The major products of lipid oxidation are hydroperoxides, which decompose into a wide range of carbonyl compounds, hydrocarbons, aldehydes, ketones and other compounds causing rancidity (Frankel 1991). Involvement of peroxidase activity in off-flavour development has been reported by Lee and Hammes (1979). Richardson and Hyslop (1985) have reported that like other heme proteins, peroxidase catalyzes the peroxidative degradation of unsaturated fatty acids yielding volatile and flavourful carbonyl compounds that may contribute to oxidized flavour.

Peroxidase (POD) is an oxidoreductase that catalyzes the oxidation of various electron donors with H_2O_2 and carries a b-type heme as prosthetic group. It is widely distributed among higher plants and has various functions. Physiological functions of peroxidase in plants involve participation in lignin synthesis, metabolism of indole-3-acetic acid, defence against infection, protection of cells from oxidative damages by H_2O_2 etc. (Gazaryan et al. 1996; Kobayashi et al., 1996).

Palm oil is the second largest edible oil in the world, which is derived from oil palm fruit. Presence of lipoxygenase and peroxidase in the fruit indicate that these may be involved in the oxidation of fat/oil, which is present in large quantities in the fruit. No information is available about the peroxidase from oil palm fruit. In this context, it would be interesting to study the biochemical properties of peroxidase from fruit. The objective of the study was to purify peroxidase from oil palm fruit and to evaluate its kinetic parameters.

Materials and Methods

Plant material : Fresh oil palm (*E. guineensis* Jacq.) fruits of *'tenera'* variety were used for the experiments and were obtained from the Central Plantation Crops Research Institute (CPCRI), Palode, Thiruvananthapuram. The fruits were thoroughly washed before use.

Preparation of acetone powder : The exocarp of the fruit was carefully removed using a knife and the mesocarp was sliced into small pieces. The mesocarp (100 g) was homogenised using a mortar and pestle and then extracted with a total volume of 1 L acetone chilled to -20°C. Acetone extract was filtered and the extraction of the residue continued (about 5 times each with 200 ml) until the filtrate was clear. Residue obtained after the filtration of acetone was dried at room temperature for 4 h and stored in desiccater at 4°C.

Extraction of peroxidase : Acetone powder of the mesocarp (50 g) was suspended in 1 L 0.1 M phosphate buffer, (pH 7.0) containing 1% polyvinylpyrrolidone (PVP) and POD was extracted by stirring for 1 h at 4°C. This was filtered through two layers of cheesecloth and the filtrate was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant obtained was used as the crude enzyme preparation.

Assay of peroxidase : POD activity was determined using an assay system consisting of 20 mM guaiacol (0.5 ml), 0.1 M acetate buffer, (pH 5.0) (2.1 ml), 40 mM H_2O_2 (0.2 ml) and the enzyme extract (0.2 ml) with a final volume of 3 ml (Chance and Maehly 1995). Oxidation of guaiacol was measured by the increase in absorbance at 470 nm. One unit of enzyme activity was defined as the amount of enzyme required to increase the absorbance by 0.1/min.

Purification of peroxidase : All operations were performed at 0-4°C.

(a) *$(NH_4)_2SO_4$ precipitation :* POD obtained from acetone powder extract was precipitated with $(NH_2)_2SO_4$ at 50-95% saturation. It was kept overnight at 4°C and then centrifuged at 10,000 rpm for 30 min. The precipitate obtained was dissolved in 50 mM phosphate buffer, (pH 6.0) and dialysed against the same buffer containing 0.5 M NaCl for 22 h with 3 changes.

(b) *Sephadex G-100 chromatography :* A 2.5 cm column packed with Sephadex G-100 upto 40 cm bed height was equilibrated with 50 mM phosphate buffer, (pH 6.0) containing 0.5 M NaCl. The dialysed sample was applied to the column and 5 ml fractions at a flow rate of 20 ml/h were collected. Absorbance at 280 nm and POD activity of each fraction were measured. The fractions having peroxidase activity were

*Corresponding Author

pooled together, dialysed against 5 mM Tris-HCl buffer, (pH 8.4) for 22 h with three changes.

(c) *DEAE-Cellulose chromatography :* A 2 cm column packed upto 15 cm bed height with DEAE-cellulose was equilibrated with 5 mM Tris-HCl buffer, (pH 8.4). The dialyzed sample was applied to the column and washed with the same buffer at a flow rate of 1 ml/min. The retained enzyme was eluted with a linear gradient of 0-1 M NaCl in Tris-HCl buffer, (pH 8.4) (150 ml + 150 ml) at the same flow rate. Fractions (3 ml) were collected on which the absorbance at 280 nm and peroxidase activity were determined. Fractions with POD activity were pooled together, dialyzed against 0.1 M acetate buffer, (pH 5.0) and used for further characterization studies.

Effect of pH : The optimum pH for POD activity was determined by using buffers of pH values 2 - 10 employing the above assay procedure. Buffers used were, for pH 2 and 3, Glycine-HCl buffer; (pH 4 and 5), Acetate buffer; (pH 6 and 7), Phosphate buffer, (pH 8), Tris-HCl buffer (pH 9 and 10), Glycine-NaOH buffer (pH 10).

Effect of substrate concentration : Activity of peroxidase at varying concentrations of guaiacol, pyrogallol, ABTS and H_2O_2 were determined and K_m values were calculated from Lineweaver-Burk plot.

Thermal stability : Thermal stability experiments were performed by subjecting the enzyme extract to heating at 30-100^{0}C. The enzyme extract (2 ml) was taken in separate test tubes and kept at respective temperatures for 60 min. From each tube, an aliquot of 0.2 ml was withdrawn at 5 min intervals and cooled by immersing in ice and was assayed immediately for residual peroxidase activity.

pH stability : Enzyme extract (2 ml) in different tubes were adjusted to pH values 2-10 by adding 1M HCl or 1M NaOH. Each sample was kept for 30 min at ambient temperature (30-32^{0}C). At the end of the experimental period, the pH was adjusted back to initial pH 5.0 and the residual enzyme activity was assayed as before.

Indole-3-acetic acid (IAA) oxidase assay : IAA oxidase activity was determined by spectrophotometric measurement at 247 nm. The standard reaction mixture consisted of 0.5 mM IAA (0.5 ml), 0.1 mM H_2O_2 (0.2 ml), 0.1 mM dichlorophenol (0.1 ml), 0.1 M acetate buffer, (pH 5.0) (2.15 ml), and the enzyme extract (0.05 ml) in a total volume of 3 ml. The reaction was started by adding the enzyme extract and the increase in absorbance at 247 nm was measured (Thomas et al. 1980). One unit of enzyme activity was defined as the amount of enzyme required to increase the absorbance by 0.1/ min.

Polyacrylamide gel electrophoresis : Electrophoresis was performed on polyacrylamide gels having a resolving gel composition of 7.5% and stacking gel composition of 5%. A constant power supply of 20 mA/plate was employed. The reservoir buffer was Tris-glycine buffer having a pH of 8.8. Peroxidase bands were detected by immersing the gels in a solution of acetate buffer, (pH 5.0) containing 1% benzidine and 0.3% H_2O_2 (Demetri and James 1979). Colour development occurred within 2 min. Protein band pattern was visualised by staining with Coomassie Brilliant Blue. The gels were also stained for glycoprotein by the modified Periodic acid-Schiff (PAS) technique (Zacharius 1969).

SDS-PAGE : Electrophoresis under denaturing conditions was performed at 10% polyacrylamide gel to the discontinuous buffer system of Laemmli (1970). Electrophoresis was carried out in vertical slab gels and the runs were performed at a constant current intensity of 15 mA/plate in the stacking and 30 mA/plate in the running gels. The molecular weight markers in the range 14-66 kDa (Sigma) were also run. Protein bands were visualized by staining the gel with Coomassie Brilliant Blue.

Molecular weight determination : A 2.6 cm diameter Pharmacia column packed with Sephadex G-200 (Sigma) up to 70 cm bed height was used for the experiment. The column was pre - equilibrated with 50 mM potassium phosphate buffer, (pH 6.0) containing 0.1 M KCl. Purified enzyme (1 ml) was applied and eluted with the same buffer system at a flow rate of 15 ml/h. Fractions of 1 ml each were collected. The system was calibrated with the following M_r markers; β-amylase (200 kDa), bovine serum albumin (66 kDa) and carbonic anhydrase (29 kDa).

Determination of protein : Protein content of the samples was quantified according to the method of Lowry et al (1951) using bovine serum albumin as standard. Appropriate controls were also done for each sample.

Results and Discussion

Purification of peroxidase : Oil palm fruit POD was purified 152-fold to homogeneity with a recovery of 16% (Table 1). POD was precipitated with $(NH_4)_2SO_4$ in the range of 50-95%. The 50-95% $(NH_4)_2SO_4$ precipitate was concentrated, dialysed against 50 mM phosphate buffer, (pH 7.0) containing 0.5 M NaCl and applied to the Sephadex G-100 column. On eluting the column with the same buffer system, POD came out with the void volume, indicating a very high molecular weight for the protein (Fig. 1). Fractions having POD activity were pooled together, dialyzed against 5 mM Tris-HCl buffer, (pH 8.4) and fractionated using DEAE-Cellulose column. Major

TABLE 1. PURIFICATION PROTOCOL OF PEROXIDASE

Fraction	Volume, ml	Total activity, Units	Total proteins, mg	Specific activity, Units/mg protein	Fold purification	Yield, %
Crude extract	1000	1661	27.6	60.18	1	100.0
40-95% $(NH_4)_2SO_4$ Precipitate	2	717	3.5	204.90	3.4	43.2
Sephadex G-100 Gel filtration	5.5	650	1.9	263.51	5.7	39.8
DEAE-Cellulose Column	3.5	274.4	0.03	9146.70	151.9	16.5

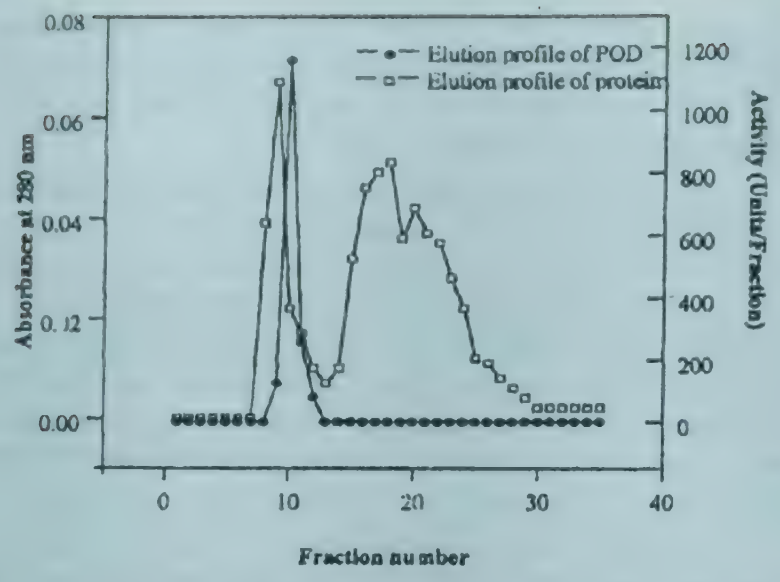

Fig. 1. Molecular exclusion chromatography of POD on Sephadex G-100

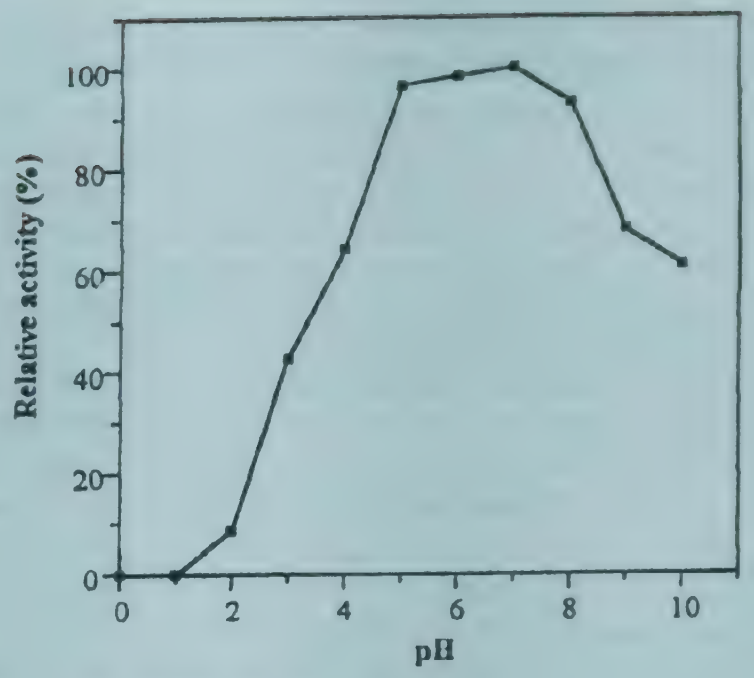

Fig. 4. pH stability of purified peroxidase

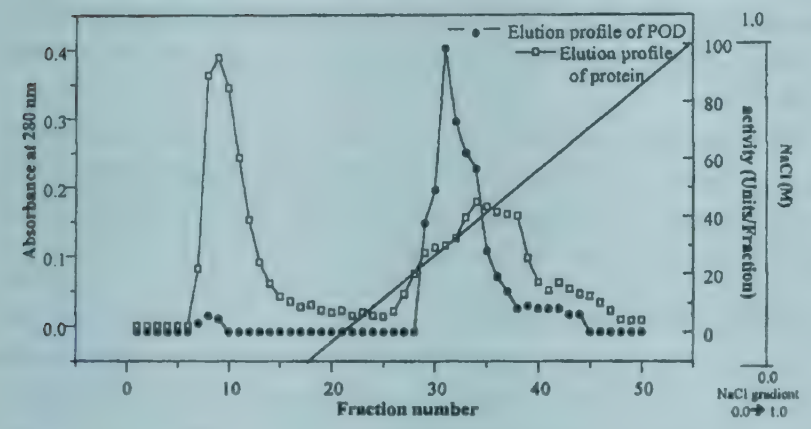

Fig. 2. Ion-Exchange chromatography of POD on DEAE-Cellulose

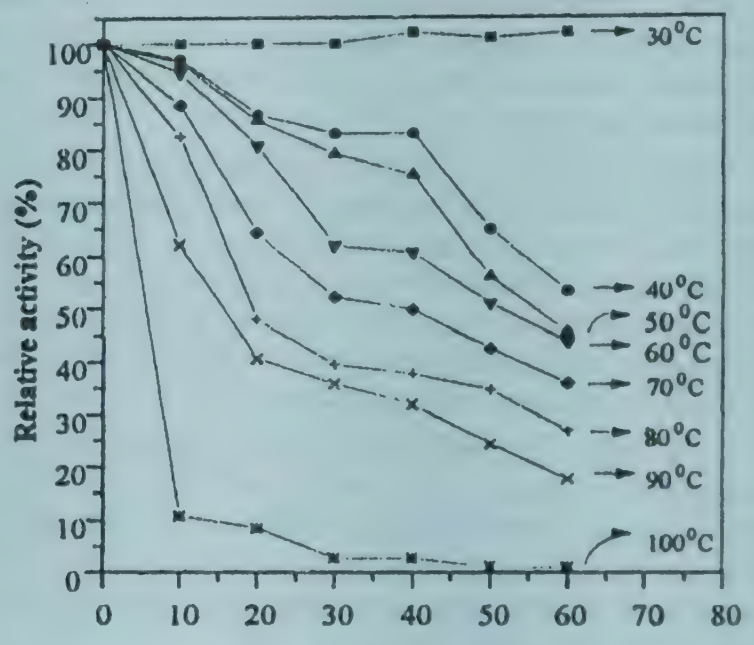

Fig. 5. Thermal stability of purified peroxidase

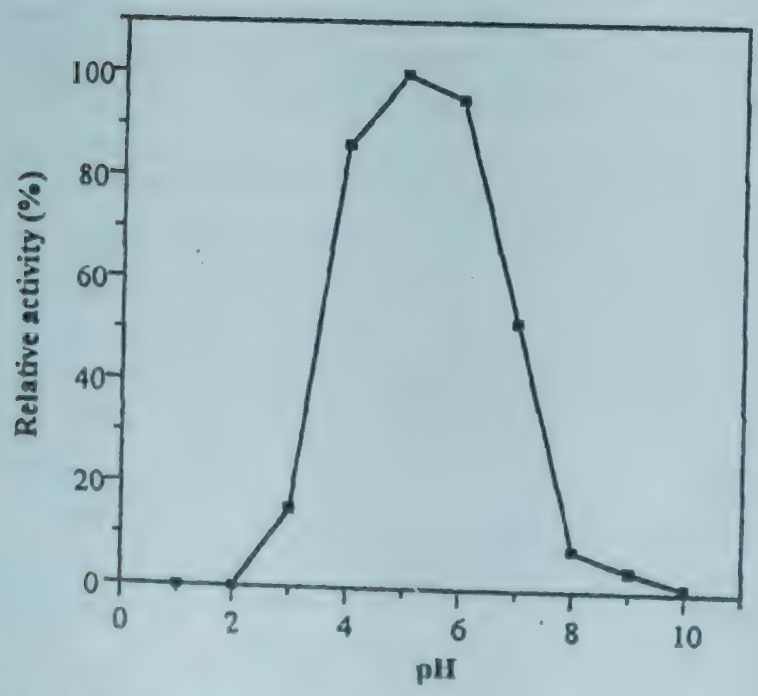

Fig 3 Optimum pH of purified peroxidase

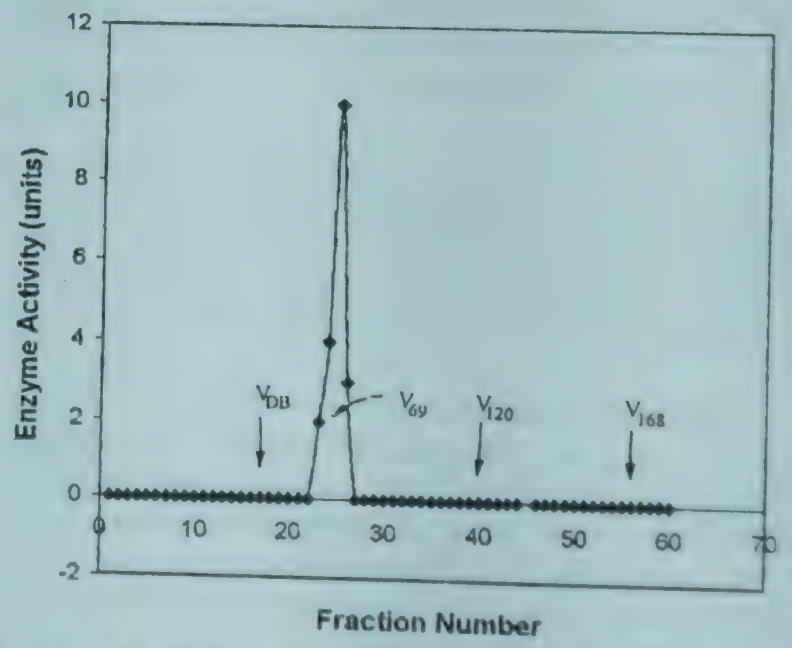

Fig. 6. Gel filtration chromatography of MPOD over Sephadex G-200

portion of the POD was bound to DEAE-Cellulose and was eluted with a linear gradient of 0.1 M NaCl in 5 mM Tris-HCl, (pH 8.4). The enzyme was eluted at a salt concentration of 0.375 M NaCl along with a major protein band (Fig. 2).

The above three steps resulted in the purification of POD to homogeneity resulting in a fold purification of 152 with a yield of 16%. This enzyme preparation was used for further characterization studies.

Characterization of purified POD : By using guaiacol as substrate, peroxidase showed a pH optimum of 5.0 (Fig 3). It exhibited greater stability with respect to H^+ ion concentration in the pH range 4.8 (Fig. 4). But as the pH was lowered below 4, a sharp decline in stability was observed and at very low pH, its activity lost completely.

The peroxidase in oil palm fruit exhibited high thermal stability. As the temperature was increased from 30^{0}C, there was a gradual reduction in activity. As the duration of exposure was increased, the loss in activity was greater. Up to 50^{0}C for 1 h, the enzyme retained 50% of the original activity (Fig. 5). POD is reported to be one of the most heat stable enzymes in plants. It was observed that 6 min at 121^{0}C was needed to inactivate peroxidase in green peas (Vamos-Vigyazo 1981). The reduction of peroxidase activity during heating was used as a measure of adequate blanching. However, the resistance to treatment depends on the source of the enzymes as well as the assay conditions, especially pH and nature of substrate employed. The present results showed that the thermal stability of oil palm fruit peroxidase was greater than that reported for cotton (Triplett and Mellon 1992), strawberry (Civello et al. 1995) and coconut (Mujer et al. 1983). It has been shown that the thermal stability of peroxidase was due to the presence of a large number of cystein residues in the polypeptide chain. It was also suggested that the non-linear inactivation curves are due to the formation of new higher thermostable complexes formed from thermally denatured enzyme protein and groups of peroxidase that remain active (Vamos-Vigyazo 1981).

Substrate concentration studies carried out using Lineweaver-Burk plot showed an apparent K_m of 2 mM for guaiacol and 1.33 mM for H_2O_2. The K_m values for the substrates ABTS and pyrogallol were 1.6 mM and 1.2 mM. The K_m values for H_2O_2 for ABTS and pyrogallol were 3.6 mM and 4.8 mM, respectively (Fig. 6). The present study has demonstrated that guaiacol is a good substrate for oil palm fruit POD, when compared with POD from other substracts.

Effect of various inhibitors : Among the compounds tested, sodium azide, dithiothreitiol and sodium metabisulphite at 1 mM concentration inhibited peroxidase activity completely. The inhibition exerted by EDTA was only marginal even at 5 mM concentrations (Table 2).

Indole-3-acetic acid (IAA) oxidase activity of peroxidase : Table 3 summarises the results of the study. The cofactors tested were Mn^{2+} and 2, 4-Dichlorophenol (DCP). From the results, it is clear that oxidation of IAA occurred only when H_2O_2 was present in the reaction medium. The only cofactor needed was DCP and the presence of Mn^{2+} retarded the reaction rate.

TABLE 2. EFFECT OF INHIBITORS ON THE ACTIVITY OF MPOD

	Concentration of activator / inhibitor	Relative activity, % MPOD
NaN_3	1 mM	0.0
	10 mM	0.0
DTT	1 mM	0.0
$Na_2S_2O_5$	0.5 mM	58.0
	1 mM	0.0
EDTA	1 mM	98.6
	5 mM	97.5

TABLE 3. INDOLE-3-ACETIC ACID ACTIVITY OF PEROXIDASE

Reaction system	IAA Oxidase activity, Units/mg protein
Buffer + IAA	0.0
Buffer + IAA + H_2O_2	1.3
Buffer + IAA + Mn^{2+}	1.3
Buffer + IAA + Mn^{2+} + H_2O_2	1.3
Buffer + IAA + DCP	3.0
Buffer + IAA + DCP + H_2O_2	29.0
Buffer + IAA + Mn^{2+} + DCP	3.6
Buffer + IAA + Mn^{2+} + DCP + H_2O_2	17.3

TABLE 4. EFFECT OF PHENOLS ON IAA OXIDATION BY MPOD

Phenolic compounds	Relative activity, % MPOD
2,4-Dichlorophenol	100.00
Resorcinol	11.54
Phenol	10.98
p-Cresol	18.46
Catechol	9.54
DOPA	5.46

Among the various phenols tested as cofactors, DCP exhibited greatest activity. Phenol and *p*-cresol, which were monohydroxyphenols and resorcinol, which is a m-dihydroxyphenol were not effective cofactors of oxidase activity as indicated by the present studies (Table 4). Catechol and DOPA, which are the o-dihydroxyphenols were also tested for their activities as cofactors. The results obtained were not encouraging. Anionic POD from orange flavedo exhibited IAA oxidase activity in the presence of H_2O_2 and DCP, but was inhibited by Mn^{2+} (Chamarro and Molina 1989). For tomato peroxidase, the reports are conflicting. According to one report, oxidation of IAA is negligible, unless the reaction mixture is supplemented with H_2O_2 (Kokkinakis and Brooks 1979), while the other says that a decline in enzyme activity occurs in the presence of H_2O_2 (Jen et al. 1980). But for oil palm fruit peroxidase, H_2O_2 was needed for its oxidative activity.

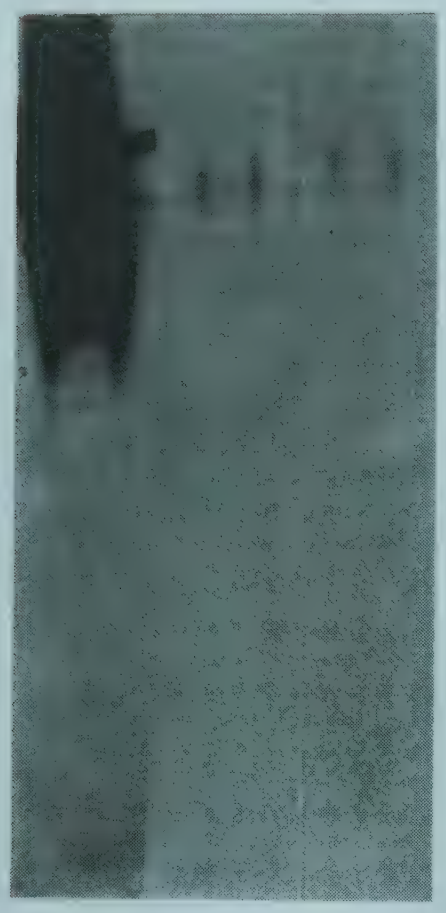

Plate 1 Native PAGE of purified peroxidase

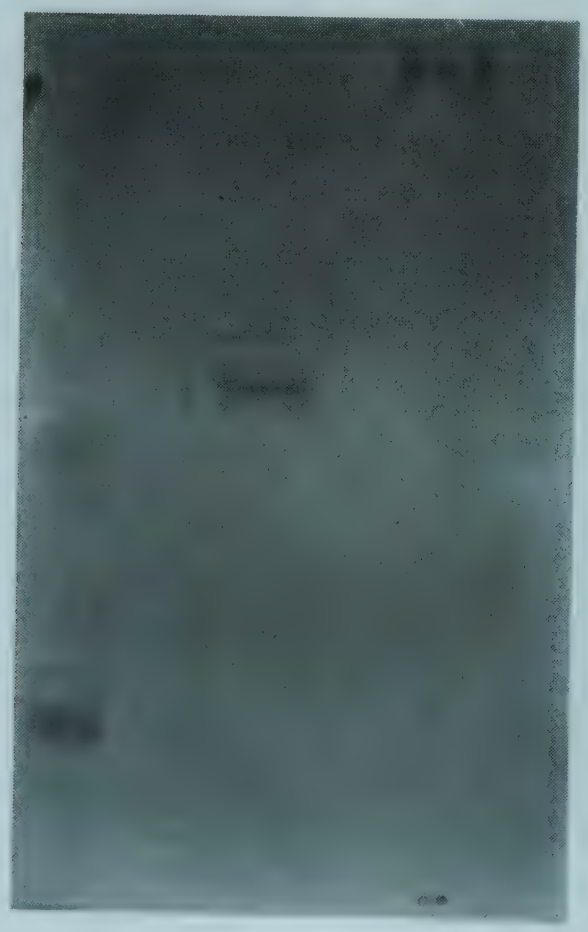

Plate 2 SDS-PAGE of purified peroxidase

Electrophoresis : Purified enzyme on native PAGE revealed a single band for POD activity (stained with benzidine) and a single band for protein. This confirmed that the enzyme preparation was pure. Glycoprotein nature of POD was confirmed by staining the gel with PAS reagent after PAGE. A pink coloured band appeared at the region of enzyme activity (Plate 1).

Molecular weight of oil palm fruit POD : Purified POD on electrophoresis (SDS-PAGE) on 10% gel showed a band having a molecular weight of 48 ± 2 kDa (Plate 2). The molecular weight of the purified enzyme was also determined by gel filtration on Sephadex G-200 gel filtration column (Plate 2). POD was eluted as a single peak corresponding to an estimated molecular weight of 200 kDa. The comparison of molecular weights obtained by these two methods revealed that purified POD was a 200 kDa protein having a subunit molecular weight of 48 ± 2 kDa.

Acknowledgements

The first author thanks CSIR, India for a Senior Research Fellowship. The authors thank Director, Regional Research Laboratory (CSIR), Thiruvananthapuram for providing the laboratory facilities.

References

Chance B, Maehly, M. (1955) In : Methods in Enzymology. Colowick SP, Kaplan NO (eds), Academic Press, New York, Vol 2 : p. 764

Chamarro J, Molina I (1989) Oxidation of indole-3-acetic acid by an apparently homogeneous peroxidase from the flavedo of Washington avel oranges (*Citrus sinensis*). J. Food Biochem 13 : 361-375

Civello PM, Martinez GA, Chaves AR, Anon MC (1995) Peroxidase from strawberry fruit (*Fragaria ananassa* Duch.) : Partial purification and determination of some properties. J Agri Food Chem 43 : 2596-2601

Demetri M, Kokkinakis, L Brooks (1979) Tomato peroxidase purification, characterization and catalytic properties. Plant Physiol. 63 : 93-99

Frankel EN (1980) Lipid oxidation. Prog Lipid. Res. 19 : 1-22

Frankel EN (1991) Recent advances in lipid oxidation. J Sci Food Agri 54 : 495-511

Galliard T, Chan HWS (1980) In : Biochemistry of Plants. Stumpf PK (ed) Academic Press, New York, pp 132-157

Gazaryan IG, Lagrimini LM, Ashby GA, Thorneley RNF (1996) The mechanism of indole-3-acetic acid oxidation by plant peroxidases. Anaerobic stopped-flow spectrophotometric studies and horseradish and tobacco peroxidases. Biochem J 313 : 841-847

Hildebrand DF (1989) Lipoxygenases. Physiologia Planta 76 : 249-253

Jen JJ, Seo A, Flurkey WH (1980) Tomato peroxidase : purification via hydrophobic chromatograpy. J Food Sci 45 : 60-63

Kobayashi A, Fukusaki E, Kajiyama S (1996) In : Plant Peroxidases, Biochemistry and Physiology. Obinger C, Burner U, Ebermann R, Penel C, Greppin H (eds) University of Agriculture, Vienna and University of Geneva, Switzerland, pp 292-297

Kokkinakis DM, Brooks JL (1979) Hydrogen peroxide mediated

oxidation of indole acetic acid by tomato peroxidase and molecular oxygen. Plant Physiol 64 : 220-223

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature 227 : 680-685

Lee YC, Hammes JK (1979) Heat inactivation of peroxidase in corn-on-the-cob. J Food Sci 44 : 785-787

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193 : 265-285

Mujer CV, Menzdoza EMT, Ramirez DA (1983) Coconut peroxidase isoenzymes : Isolation, partial purification and physicochemical properties. Phytochem 22 : 1335-1340

Nawar WW (1985) In : Lipids from Food Chemistry. Fennema OR (ed) Marcel Dekker Inc., New York, pp 139-244

Richardson T, Hyslop DB (1985) In : Enzymes in Food Chemistry. Fennemm OR (ed) Marcel, Dekker, Inc New York, pp 371-476

Siedow JN (1991) Plant lipoxygenase : Structure and function. Ann Rev Plant Physiol Plant Mol Biol 42 : 145-188

Thomas RL, Morr CV, Jen JJ (1980) Oxidation of indole-3-acetic acid by homogeneous tomato fruit peroxidase. J Food Biochem 4 : 235-246

Triplett BA, Mellon JE (1992) Purification and characterization of anionic peroxidase from cotton (*Gossypium hirsutum* L.) Plant Sci 81 : 147-154

Vamos-Vigyazo L (1981) Polyphenol oxidase and peroxidase in fruits and vegetables. CRC Crit Rev in Food Sci Nutri 15 : 49-127

Zacharius RM, Zell TE, Morrison JH, Woodlock JJ (1969) Glycoprotein staining following electrophoresis on acrylamide gels. Anal Biochem 30 : 148-152

Received 13 June 2000; revised 25 June 2001, accepted 9 December 2001

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 14-20

Alkalinization Process for Cocoa (*Theobroma cacao* L.) Nibs and Quality Evaluation of the Powder by Response Surface Methodology

E.S. BISPO*[1], V.L.P. FERREIRA[1], H.C. MENEZES[1], A.A. VITALI[2], AND K. YOTSUYANAGI[2]

[1] Faculty of Food Engineering FEA, State University of Campinas UNICAMP, CP 6121, 13083-970, Sp, Brazil.

[2] Institute of Food Technology ITAL, Av. Brazil 2880, Campinas, SP, Brazil.

In order to develop a Brazilian technology, process parameters for the production of alkalinized cocoa powder from nibs were determined using a 2^3 experimental design with four repetitions at the central point giving a total of 18 treatments, thus obtaining products, which differed from one another with respect to colour, pH and flavour. The independent variables were temperature (60, 72, 90, 108 and 120^{0}C), time (30, 54, 90, 120 and 150 min) and potassium carbonate (K_2CO_3) concentration (0.0, 1.22, 3.0, 4,78 and 6.0%), with a moisture content of 45% based on the mass of nibs. Within the limits studied, Response Surface Methodology (RSM) was shown to be adequate to represent the process with respect to the responses for limiting ash content, pH, L*a*b* colour, ratio of b*/a* and the acceptance of colour and flavour for the alkalinized cocoa powders.

Keywords : Alkalinization, Nibs, Alkalinized cocoa powder, Responses Surface Methodology.

The alkalinization of cocoa products was started in 1828 by Van Houter in Holland with the aim of improving the product quality and has been widely studied ever since. In the processing of cocoa products, in addition to the processes of fermentation and roasting, which result in the development of desirable characteristics in the final product, alkalinization us used to accentuate the taste aroma by reducing the acidity and astringency and also to obtain a variety of colours from yellowish brown to reddish brown, even dark brown or black, depending on the processing conditions and type of alkali used (Cook 1972; Andres 1983; Martin et al. 1987; Kattenberg 1995). Basically, the cocoa products react with the alkalinizing aqueous solutions under different times and temperatures in the presence of O_2, when reactions occur, resulting in colour, taste and dispersibility alterations (Anonimo 1970; Schenkel 1973; Terink and Brandon 1982; Martin at al., 1987; Minifie, 1989; Anonimo 1994). In the range of alkalinization processes, Response Surface Methodology (RSM) makes it possible to obtain cocoa powders with L*, a*, b* and b*/a* colour parameters which are of great commercial importance.

The various types of alkalinized cocoa powders are used for different products, such as chocolate powders, mousses, pudding powders, cakes, coverings, ice cream and biscuit powders, making them more attractive and with more acceptable flavours, as well as offering the alternative of reducing the use of synthetic colouring agents (Kattenberg 1995; Terink and Brandon 1982)

Modifications in the various stages of the alkalinization process and the use of different alkalinization agents such as sodium, potassium, ammonium and magnesium hydroxide and potassium carbonate have been tested, varying the time/ temperature combinations to improve the process (Martin et al., 1987; Kattenberg 1995). The concentrations of each ingredient and the processing methods are specific for each manufacturer and considered to be industrial secrets or protected by patents (Minson 1989).

*Corresponding Author

Materials and Methods

Sample preparation : The nibs (fragmented cotyledons) used, were obtained from fermented roasted cocoa beans of the variety 'Forastero' from Bahia (Brazil), all within the international microbiological quality standards (AOAC 1994).

Alkalinization process : The stages of the alkalinization process were carried our using simple equipment. The actual alkalinization occurred in a jacketed heating vessel with 2 paddles, with potassium carbonate as the alkalinization agent. The internal temperature was controlled by installing a thermometer with a bimetallic shaft and scale from zero to 120^{0}C. A manometer and a pressure regulating valve were necessary to control the steam flow and a rotameter to control the air flow rate at 200 litres per hour (Fig 1).

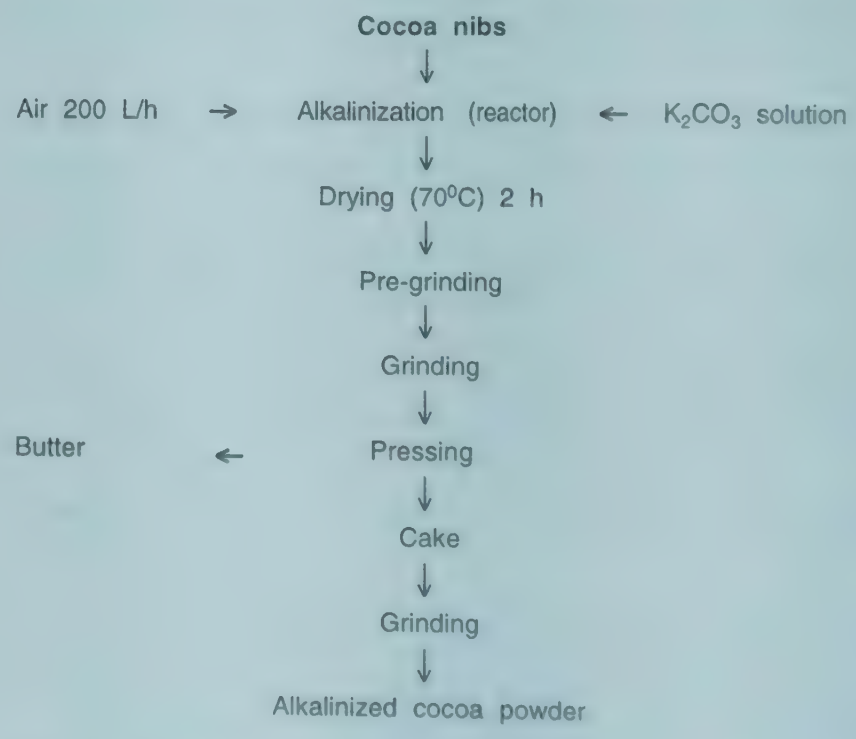

Fig. 1. Flow diagram of the alkalinization process for cocoa powder from nibs.

The moisture content was reduced in a forced air oven at 70^{0}C for 2 h, giving a final moisture content of 2%. The fat content was reduced in a manual hydraulic press (EVA) with a capacity for 100 ton/cm^2. Maximum extraction was obtained using the time x pressure binomial, increasing the pressure gradually up to 50 tone/cm^2 over a period of 45 min.

Pre-grinding was carried out using a cylinder mill (Suprema) with a 3 hp engine, and the final grinding in a cooled 3-cylinder mill (Pilon), to obtain a granulometry of 99% mesh 200 (0.75 um) after four passages through the mill with maximum pressure on the rolls.

Parameters selected, their levels and coding: The ranges of factors and levels were based on preliminary tests and the literature for each factor. The ranges of experimental conditions (levels) were defined as follows:

Temperature: 60 to 120^{0}C ; Time: 30 to 150 min; and potassium carbonate concentration of 0 to 6%, this being the agent which gave the best colours and flavours in the preliminary tests.

Experimental design: The alkalinization process was based on an experimental design for Response Surface Methodology (Thompson 1982), of the central compound rotational type with 18 treatments (8 factorial points, 6 axial and 4 repetitions of the central point), in the ranges of experimental interest (levels) (Table 1). The pH and L*a* b* colour were also analysed in two commercial brands of cocoa powder.

Analysis of the alkalinized cocoa powders

Ash content - Sample (5g) was placed on a porcelain tray previously heated in a muffle furnace to 600^{0}C, cooled to ambient temperature and weighed. The sample was dried in a stove and carbonized at low temperature followed by ashing in a muffle furnace at 600^{0}C. The sample was cooled to ambient temperature and weighed. Successive operations of heating and cooling were carried out to constant weight (AOAC 1994).

pH value - The sample (10g) was weighed and 90 ml boiling water was slowly added with stirring. After filtering, the filtrate was cooled to 20-25^{0}C and the pH was immediately determined using a potentiometer standardised with buffers at pH 4.00 and 6.86 (AOAC 1994).

Colour - The colour was determined in a tristimulus Colour QUEST II spectrophotometer (Hunter colorimeter, developed in 1952) with Reflectance Specular included (RSIN) and also the CIELab system (CIE= Commission International de l'Eclairage, 1931; L = lightness; a = redness; b =yellowness.) AT 10^0 and using the D65 illuminant.

Sensory evaluation - The products obtained were subjected to an acceptance test in the form of chocolate powders (1.5% alkalinized cocoa powder and 8% sugar in skimmed milk), with 30 judges using a 9-point Hedonic scale (1 - dislike extremely and 9 - like extremely). The flavour evaluation was conducted in booths with red light to mask the appearance of the sample, and the colour test was conducted outside the booths using natural light.

Statistical analysis - The means of each of the 18 treatments for each response were analysed using Response Surface Methodology (RSM), using multiple regression, adjusting 2nd order mathematical models, containing linear and quadratic terms and interactions of the three variable independents. Bi-dimensional graphs were drawn using the programme STATISTICA version 5.0, fixing one of the factors or variable independents at the central point (Thompson 1982).

TABLE 1. EXPERIMENTAL DESIGN FOR THE INDEPENDENT VARIABLES

Treatment	T^{0}C	T, min	K_2CO_3, %
1	72	54	1.22
2	108	54	1.22
3	72	126	1.22
4	108	126	1.22
5	72	54	4.78
6	108	54	4.78
7	72	126	4.78
8	108	126	4.78
9	60	90	3.00
10	120	90	3.00
11	90	30	3.00
12	90	150	3.00
13	90	90	0.00
14	90	90	6.00
15	90	90	3.00
16	90	90	3.00
17	90	90	3.00
18	90	90	3.00

Results and Discussion

Table 2 presents ash, pH, L*, a*, b* and b*/a* colour values from alkalinized cocoa powders and the acceptance of colour and flavour of the chocolate beverages.

Ash content: Ash = 10.4802 - 0.4098 x - 5.0188 y - 0.3873 y^2 + 1.1900 x.z + 0.7850 x.y + 1.1450 y. $R^2 = 0.95$(1)

where T = x; K_2CO_3 = y and t = z

According to Brazilian law, the maximum ash content permitted in alkalinized cocoa products is 14% ABIA (1992). Fig 2 shows that the use of potassium carbonate (K_2CO_3) at 5.95% and 4.45% with temperatures of 60.35^{0}C and about 116.8^{0}C respectively, resulted in acceptable levels of ash, below 14%. Fig 3 shows the acceptable ash content in the range of potassium carbonate (K_2CO_3) concentrations with time studied.

pH value - pH = 7.3528 - 0.2375 a + 1.0697 b - 0.1887 ab

R^2 = 0.95 (2)

where T = x; K_2CO_3 = y and t = z

Figs 4 and 5 show that only the potassium carbonate (K_2CO_3) concentration and the temperature influenced the pH of the powders at $p \leq 0.05$ if one considers the range of potassium carbonate (K_2CO_3) concentration from 0.32 to 3.0%.

TABLE 2 ASH, pH L*, a*, b* AND b*/a* COLOUR VALUES FROM ALKALINIZED COCOA POWDER AND ACCEPTANCE OF COLOUR AND FLAVOUR OF THE CHOCOLATE BEVERAGES.

Treatment	Ash, g%	pH	Colour L*	Colour a*	Colour b*	b*/a* Ratio	Flavour acceptance	Colour acceptance
1	10.67	6.39	32.03	15.69	14.08	0.89	5.56	6.10
2	7.20	6.21	31.63	15.73	13.91	0.88	5.37	5.74
3	7.48	6.45	32.28	17.22	15.24	0.88	5.22	5.72
4	7.35	6.33	26.75	12.61	10.00	0.79	6.50	5.87
5	12.72	8.95	20.94	11.41	5.29	0.46	7.97	7.70
6	11.78	7.84	20.56	7.34	3.40	0.46	7.31	7.19
7	12.74	8.69	21.15	11.24	5.42	0.48	7.50	6.94
8	13.26	8.00	19.14	5.28	2.63	0.50	6.91	6.97
9	10.15	7.87	24.70	16.12	9.59	0.59	6.94	7.12
10	10.90	7.19	19.70	6.24	3.32	0.53	6.31	6.13
11	9.75	7.43	25.55	14.85	9.76	0.65	6.72	6.37
12	10.60	7.33	25.70	15.61	10.48	0.67	7.23	6.75
13	4.88	5.37	33.13	15.04	15.32	1.01	4.59	5.32
14	14.56	9.24	19.84	9.49	3.94	0.41	7.28	6.87
15(C)	10.42	7.35	25.87	16.02	10.20	0.63	6.72	6.94
16(C)	10.46	7.39	27.03	16.63	11.40	0.68	5.82	5.83
17(C)	10.29	7.02	24.98	14.83	9.26	0.62	7.25	7.03
18(C)	10.69	7.30	25.42	14.26	9.41	0.65	6.88	6.12

(C) repetitions at the central point

From Fig 4, it can be observed that the pH values at a temperature of 60.31^{0}C, varied from 6.16 to 8.0. Fig 5 shows that the time periods used in the processes did not influence the pH values of the alkalinized powders.

Colour parameter L* - L* = 26.25576 - 1.2249 x - 4.6345 y - 1.1889 x^2

R^2 = 0.93 (3)

where T=x; (K_2CO_3) = y and t=z

The parameter L* was considerably influenced by the potassium carbonate (K_2CO_3) concentration, it being seen that at values above 3%, the higher the potassium carbonate (K_2CO_3) concentration the lower the values for L* (darker powders). In the range from 4.45% to 5.9% potassium carbonate (K_2CO_3) and temperatures of 60.31^{0}C and 116.8^{0}C, respectively (Fig 6), the powders were darker with an L* value of 19.3. In commercial samples of alkalinized cocoa powders,

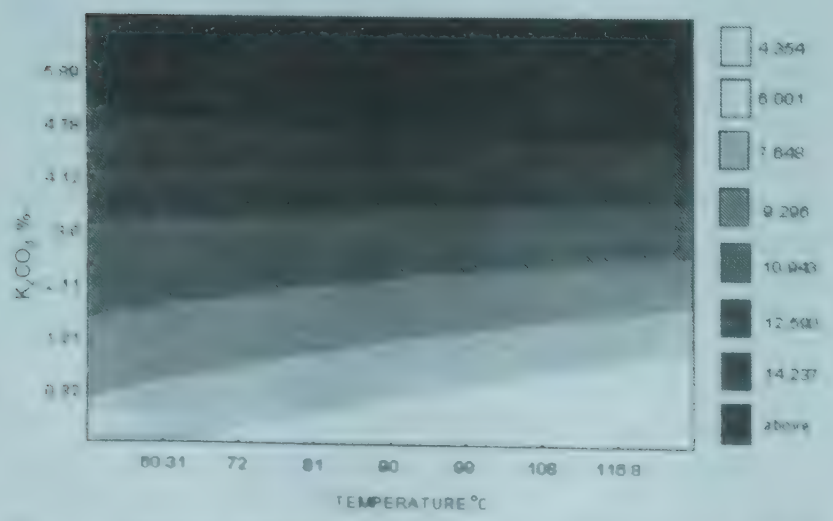

Fig. 2. Effect of temperature and K_2CO_3 concentration on the ash content of alkalinized cocoa powders

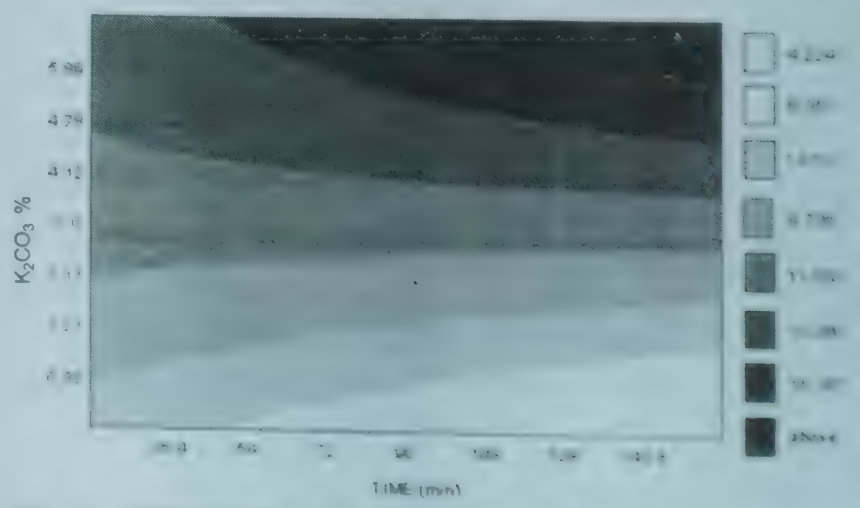

Fig. 3. Effect of time and K_2CO_3 concentration on the ash content of alkalinized cocoa powders

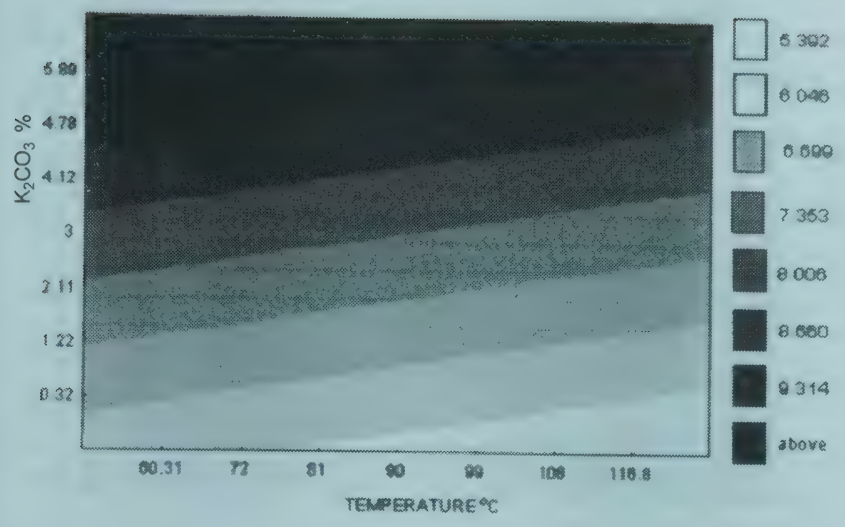

Fig. 4. Effect of independent variables temperature and K_2CO_3 concentration on the pH of alkalinized cocoa powders

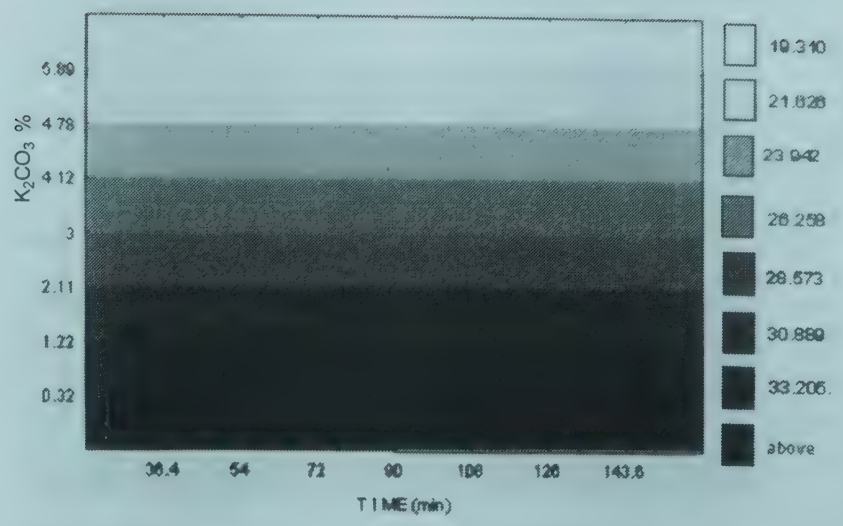

Fig. 7. Effect of the independent variables Time and K_2CO_3 concentration on the colour L* of the alkalinized cocoa powders

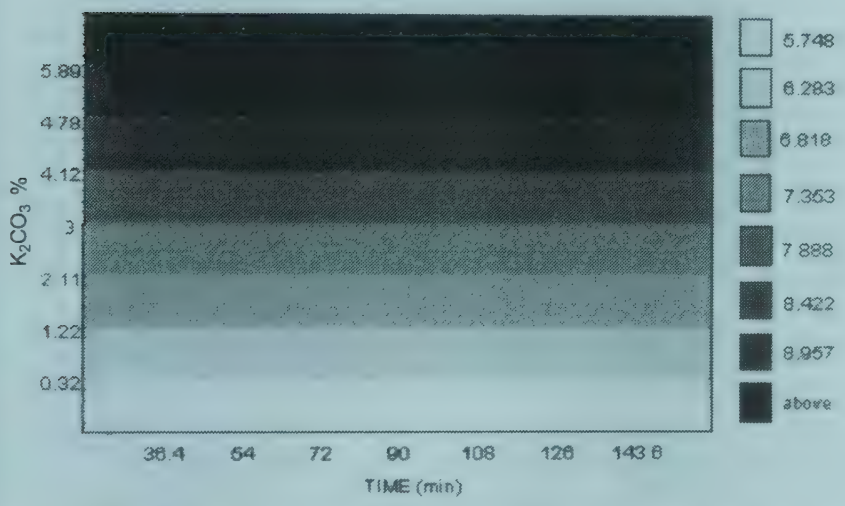

Fig. 5. Effect of independent variables Temperature and K_2CO_3 concentration on the pH of alkalinized cocoa powders

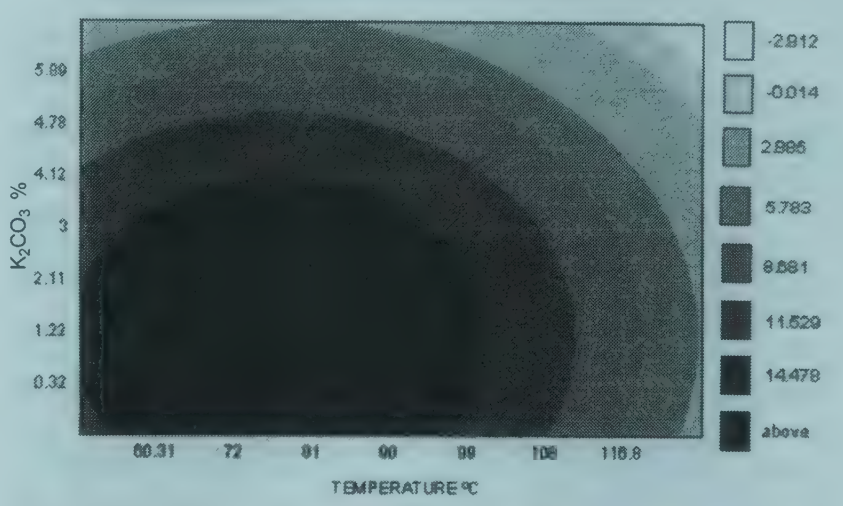

Fig. 8. Effect of the independent variables Temperature and K_2CO_3 concentration on the colour a* of alkalinized cocoa powders

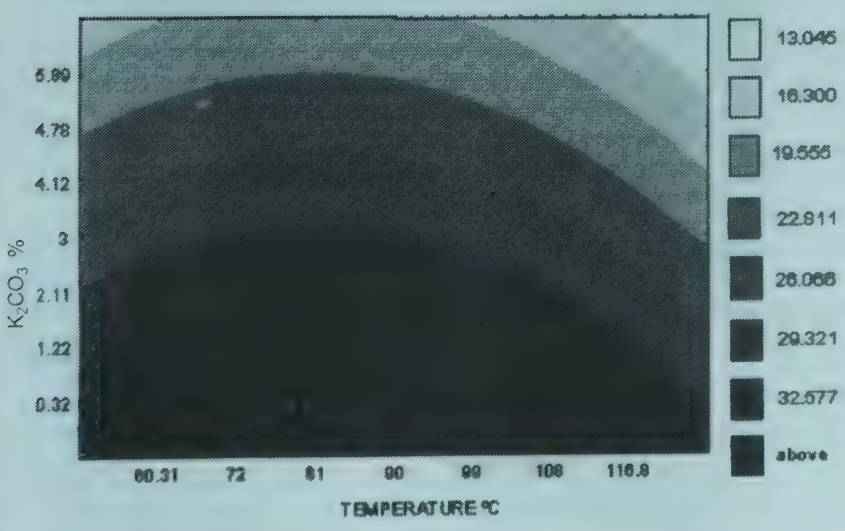

Fig. 6. Effect of the variables temperature and K_2CO_3 concentration on the colour L* of the alkalinized cocoa powders

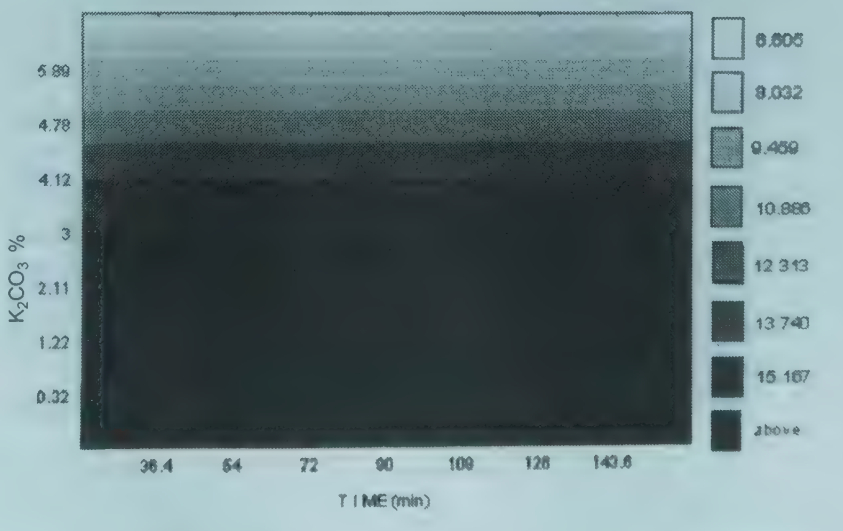

Fig. 9. Effect of independent variables Time and K_2CO_3 concentration on the colour a* of alkalinized cocoa powders

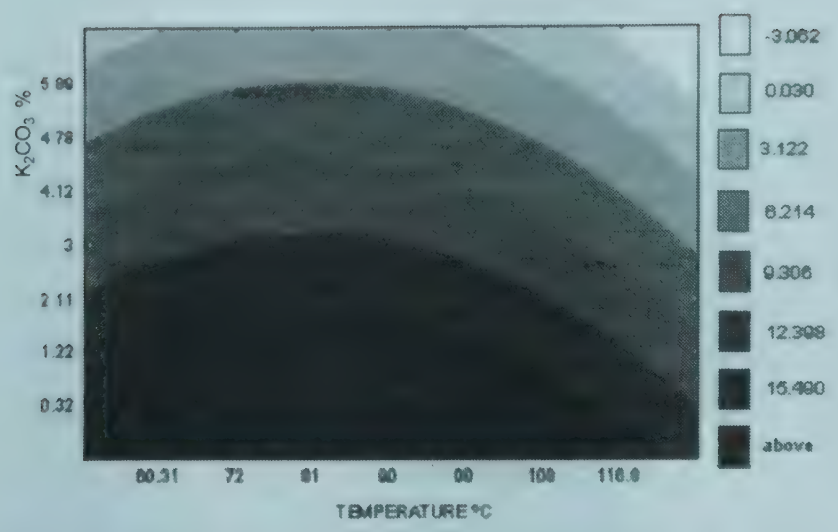

Fig. 10. Effect of the independent variables Temperature and K_2CO_3 concentration on the colour b* of alkalinized cocoa powders

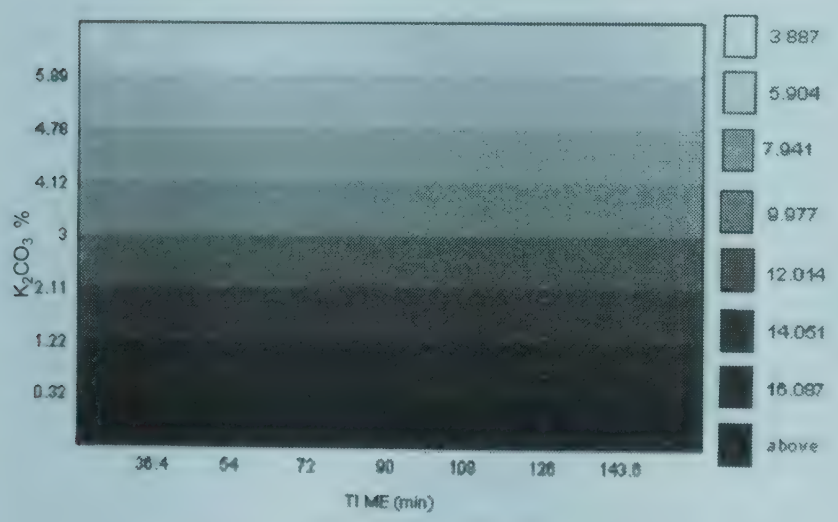

Fig. 11. Effect of the independent variables Time and K_2CO_3 concentration on the colour b* of alkalinized cocoa powders

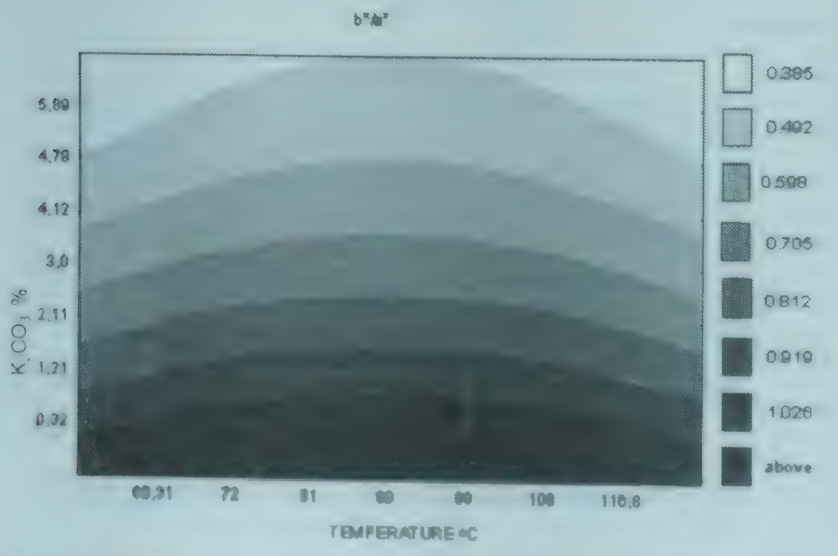

Fig. 12. Effect of the independent variables Temperature vs K_2CO_3 concentration on the colour ratio b*/a* of alkalinized cocoa powders

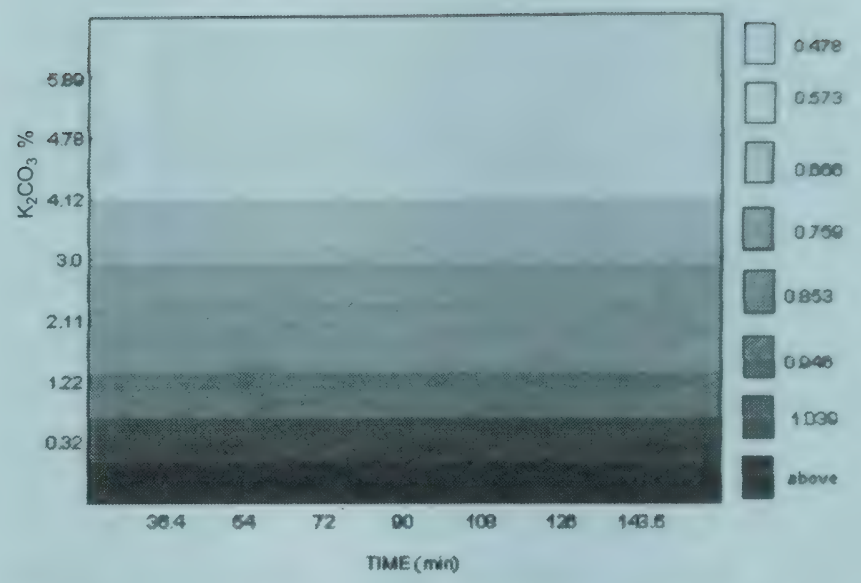

Fig. 13. Effect of the independent variables Time vs K_2CO_3 concentrations on the colour ratio b*/a* of alkalinized cocoa powders

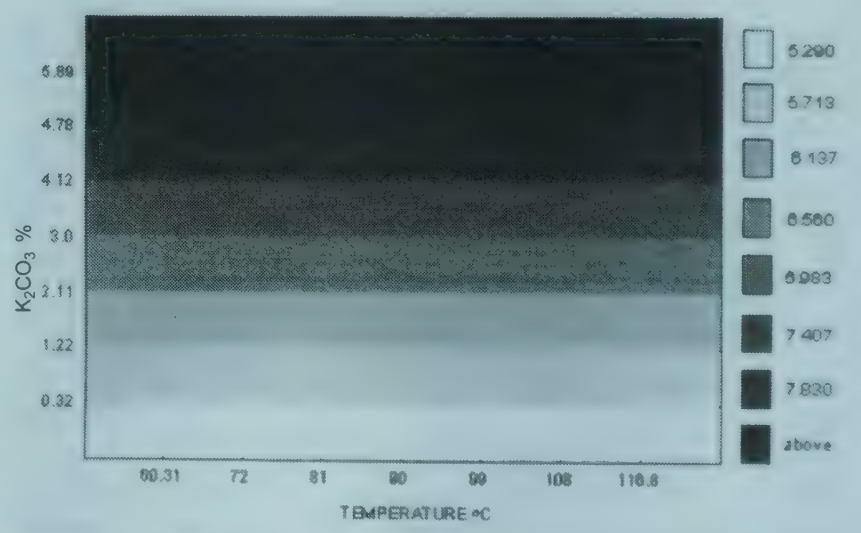

Fig. 14. Effect of the independent variables Temperature and K_2CO_3 concentration on the acceptance test for chocolate powders

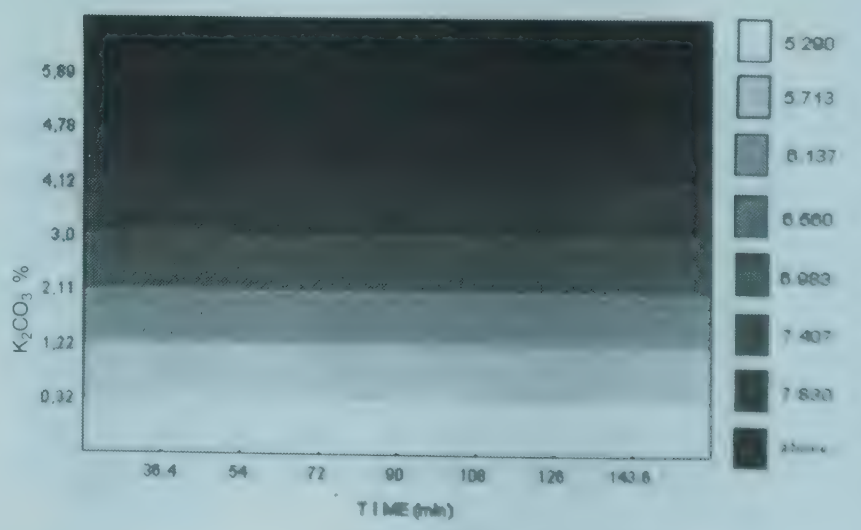

Fig. 15. Effect of the independent variables Time and K_2CO_3 concentration on the acceptance test for chocolate powders

luminosity values of 24.8 and 25.5 were obtained, values which can be obtained in the process under study. Fig 7 shows that the time periods used in the processes did not influence the L* values of the alkalinized powders.

Colour parameter a (red)* - $a^* = 15.2328 - 2.2857\,x - 2.5858\,y - 1.6042\,x^2 - 1.2206\,y^2$

$R^2 = 0.87$ (4)

where $T = x$; $K_2CO_3 = y$ and $t = z$

Increases in the concentration of potassium carbonate (K_2CO_3) and temperatures above 85^{0}C, resulted in decreases in the values for a* (red) (Fig 8), redder powders (a*>15.5) being obtained in the range from 0 to 3% Potassium Carbonate (K_2CO_3) concentration and a temperature of about 75^{0}C. Values for red between 13.35 and 14.46 (commercial samples) were obtained with approximately 3.52% Potassium Carbonate (K_2CO_3) and a temperature of about 106^{0}C. The values for a* did not vary as a function of the potassium carbonate concentration with time (Fig 9)

Colour parameter b(yellow)* - $b^* = 9.9773 - 1.5109x - 4.0773y - 1.2406x^2$

$R^2 = 0.93$ (5)

where $T = x$; $K_2CO_3 = y$ and $t = z$

The higher the potassium carbonate (K_2CO_3) concentration, the lower the values for b* (less yellow), the highest values for b* being obtained with potassium carbonate (K_2CO_3) concentrations below 2.11% and temperatures between 60.31^{0}C and 96^{0}C (Fig 10). Temperatures above 96^{0}C resulted in a decrease in the values for b*. Theoretically the values for b* of the commercial samples (7.65 and 9.78) could be produced under the conditions of this experiment at potassium carbonate concentrations between 0% and 5.6% and a temperature of about 116.8^{0}C. No significant effect ($p \leq 0.05$) of the potassium carbonate concentration with time (Fig 11) on the values for b* could be observed.

Ratio of the colours b/a** - The reddish brown colour is of great commercial importance (Terink and Brandon 1982), and the ratio of b*/a* is widely used to characterize the colour of alkalinized cocoa powders. Values greater than 0.7 are characteristic of orange brown cocoa powders which are only slightly alkalinized, and a ratio below 0.6 is characteristic of a cocoa powder of medium alkalinization and a very reddish brown colour, highly desirable in products which use alkalinized cocoa powders. (Terink and Brandon 1982).

$b^*/a^* = 0.65 - 0.02\,x^2 - 0.18\,y + 0.02\,y^2$

$R^2 = 0.93$ (6)

where $T = x$; $K_2CO_3 = y$ and $t = z$

The ratio b*/a* is influenced by the linear and quadratic potassium carbonate (K_2CO_3) concentration and by the quadratic temperature, there being no significant effect of time at $p \leq 0.05$. Under the process conditions for alkalinization used in this study, it can be seen from Fig. 12 and Fig. 13 that is possible to obtain the orange brown powders with a b*/a* ratio above 0.7, indicating only slight alkalinization, in the range of potassium carbonate concentration from 0% to approximately 2.6% and at temperatures below 60.31^{0}C and above 116.8^{0}C. Concentrations greater than 3.5% in the temperature interval studied resulted in powders with a b*/a* ratio below 0.6, and the lower this value, the lower the values for luminosity (L*), the powders becoming increasingly darker.

Colour acceptance :

$Colour = 6.5600 + 0.8567\,y$

$R^2 = 0.70$ (7)

where $T = x$; $K_2CO_3 = y$ and $t = z$

The region for colour acceptability (Fig. 14) as from "liked moderately", occurred for potassium carbonate (K_2CO_3) concentrations greater than 2.8%, covering practically the whole temperature range studied. The region of greatest colour acceptance ("liked a lot" to "liked extremely") was defined for potassium carbonate concentrations above approximately 4.4%. Fig. 15 shows that the time periods used in the processes did not influence the colour acceptance of the alkalinized powders.

Flavour Acceptance - $Flavour = 7.1549 - 0.4571\,x + 0.1904\,y - 0.52262\,x^2 + 0.1631\,z^2 + 0.1087\,xy$

$R^2 = 0.54$ (8)

where $T = x$; $K_2CO_3 = y$ and $t = z$

Treatment No. 10 (120^{0}C x 90 min. x 3.0% potassium carbonate (K_2CO_3) was evaluated as "disliked moderately" and the rest were found in the region of acceptability from "linked moderately" to "liked extremely".

Conclusions

Alkalinized cocoa powder from nibs under different process conditions were prepared using a simple equipment. The mathematical models obtained for the variables studied were shown to adequately represent the process within the experimental limits used for potassium carbonate concentration and temperature for the responses of pH, colour (L*a*b*) and the ratio b*/a* of the alkalinized cocoa powders. The time range studied did not influence the process. In the ranges of temperature and potassium carbonate concentration studied, alkalinized cocoa powders were obtained within the limits prescribed by the Brazilian legislation for the potassium carbonate and ash contents, showing b*/a* colour ratios are of great commercial importance. The colour acceptance of the chocolate powders was greatly affected by the potassium carbonate concentration, the greater the concentration i.e., the greater the acceptance of these increasingly darker powders. Treatment No. 10 was evaluated as "disliked moderately" and the others were found in a region of the scale between "liked moderately" and "liked extremely".

Acknowledgement

This project was sponsored by Fundacao de Apoio a Pesquisa do Estado de Sao Paulo (FAPESP)

References

Andres. C. (1983) New cocoa powder offers improved flavor, Food Processing, 44(3) : 36-37

Anonimo. (1970) Cocoa powder composition and nutritive value Gordian : Kakaopulves, 70 : 170-172.

Anonimo. (1994) Description del proceso de produccion de granos de cacao alcalinizado y esterilizado y sus produtos derivados cacao en polv y manteca de cacao, a partir de habas de cacao. Research Disclosure, 365 : 363-367

AOAC. (1994) Official Methods of Analysis. Association of Official Analytical Chemists. 16 edn., Washington DC

ABIA (1992) Associacao Brasileira das Industrias da Alimentacao. Compendio da legislacao de alimentos : consolidacao das normas e padroes para alimentos. 5. rev. Sao Paulo.

Cook, RL (1972) Chocolate Production and Use. 1st edn. Books for Industry, Inc, New York.

Kattenberg, RH (1995) The application of cocoa powder in chocolate confectionery. The Manufacturing Confectionery 3 : 73-83

Martin, J.R. Chichester, C.O. Mrack, E.M.; Scaweigert, B.S. (1987) In : Chocolate, Advances in Food Research Academic Press, San Diego pp 211-342

Minifie, BW (1989) Chocolate, Cocoa and Confectionery Science and Technology, 3rd edn, New York, p. 904.

Minson, E. (1992) Chocolate manufacture beans through liquor production. Manufacturing Confectioner 72, (11) : 61-67

Schenkel, H.J. (1973) Colour formation in the Dutch processing of cocoa. Manufacturing Confectioner 53, (8) : 26-28, (30) : 32-33, (10) : 44

Terink, JL, Brandon, M.J. (1982) Alkalized cocoa powders and foodstuffs containing such powders. Cacaofabrik de Zaam B U; Int Cl[3] A23G 1/00; A23G1/02 NL. 8102377. 14 maio 1981. Patent 4,435,436. 6 marco

Thompson, DR (1982) Response surface experimentation. Food Process Preserv, Westport pp 155-188

Received 25 August 2000; revised 27 June 2001; accepted 9 December 2001

ANNOUNCEMENT

The last date for receiving the nominations for FELLOWS and other awards has been extended to 30, April 2002.

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 21-25

Characterization of Cheese Whey and its Suitability for Ethanol Production by Alginate Entrapped Yeast Cells

B.S. SOOCH AND R.S. SINGH*

Department of Biotechnology, Punjabi University, Patiala - 147 002. India

Ethanol production from cheese whey using immobilized *Kluyveromyces marxianus* NCDC 39 was attempted. The periodic analysis of cheese whey showed that the amount of total solids, biochemical oxygen demand, chemical oxygen demand of the tested samples were much higher than the federal permissible limits governing the discharge of industrial effluents in India. Gel entrapment method of immobilization was selected for immobilization of yeast culture. Clarified cheese whey was found to be the suitable substrate as compared to the unclarified whey for ethanol production. To optimize the ethanol production in clarified cheese whey, various process parameters like size of inoculum, pH, incubation time, agitation were monitored.

Keywords : Cheese whey, Ethanol, Fermentation, Immobilization, Optimization, Yeast culture.

Whey is produced in huge amount in dairy industry and its disposal requires a large amount of capital investment. Approximately 85% of the total milk used for manufacturing cheese/*paneer* or *channa* is discarded as whey (Kosikowski 1978). The type and composition of whey at dairy plants depend upon the type of cheese manufactured and on the process technology employed for its production. The acid whey results from the direct acidification of milk to pH 5.1 or below. However, the sweet whey results from the rennet type enzymes at pH 5.6. Most of the milk plants do not have proper treatment system for the disposal of whey. Based on the whey constituents, many conventional uses of it, have been reported (Gandhi 1984; Marwaha and Kennedy 1988). The dumping of whey constitutes a significant loss of potential food and energy. It is also a serious pollutant as it imposes a high biochemical oxygen demand of 30,000 - 50,000 mg/litre (Marwaha and Sethi 1984). The high BOD and COD loaded whey is either released into water bodies or on land with or without prior treatment. It affects the physical and chemical structure of the soil and ultimately, it decreases the crop yield (Fritzscha 1986). The disposal of whey in the water streams or in the sea has also been considered objectionable as it affects the aquatic life while depleting the dissolved oxygen of water (Wheatland 1974; Tiwana 1985). The disposal of whey without treatment contributes immensity towards high BOD and COD of effluent of dairy industry. The federal permissible limits for the disposal of industrial effluents in India are BOD (30 mg/l), COD (250 mg/l), total solids (2600 mg/l) and pH (5.5-9.0). Although, advanced novel approaches for the utilization of whey have been developed, half of the total whey produced is discarded on the land or into water systems (Zall 1984). The lactose and proteins in whey are potential biochemical sources. However, presently, the utilization of whey for ethanol production is the area of major interest. Currently, increasing interest has been focussed on the utilization of whole cell immobilization techniques instead of immobilized enzymes. The applications of immobilized cells in alcohol production has developed very rapidly in the recent years and majority of the reports are on ethanol fermented beverages (Navarro et al 1976). The present study was carried out to find the suitability of cheese whey for ethanol production using immobilized yeast cells in flask culture at bench scale.

* *Corresponding Author E-mail : rssingh@pbi.ernet.in*

Materials and Methods

Organism : Kluyveromyces marxianus NCDC 39 was procured from National Dairy Research Institute, Karnal, India. The culture was maintained on Potato Dextrose Agar (PDA) slants containing potato extract (20%), dextrose (2%) and agar-agar (2.5%) adjusted to pH 5.5 by sub-culturing fortnightly.

Procurement of cheese whey : Fresh cheese whey was periodically collected in air tight plastic containers (5 L) from Verka Milk Plant, Patiala, India. The samples were stored at 4 ± 1^0C, until further use.

Clarification of whey : The clarification of whey was carried out by the method of Robinson et al (1976).

Immobilization of yeast culture : The method of Marwaha et al (1988) with slight modifications was used for the immobilization of *Kluyveromyces marxianus* NCDC 39.

Preparation of starter : Kluyveromyces marxianus NCDC 39 was grown in potato dextrose broth (50 ml contained in 250 ml capacity conical flasks) containing potato extract (20%, v/v), dextrose (2.0%, w/v) and adjusted to pH 5.5. The flasks were incubated at 30 ± 2^0C for 48 h under stationary conditions. The cells were harvested by centrifugation at 5000 rpm for 20 min at 4^0C and then were used for immobilization.

Entrapment of yeast cells in sodium alginate : The centrifuged yeast cells were thoroughly mixed with 2.5% (w/v) sodium alginate, unless otherwise specified. The resultant yeast-alginate slurry was extruded as drops into calcium chloride solution (0.075 M) at room temperature. To obtain the beads of uniform size (average diameter 3.0 mm), the slurry was extruded through a sterilized glass syringe (20 ml capacity) fitted with a 20 gauge needle. The beads were left suspended in calcium chloride solution (0.075 M) for 24 h to allow complete gelation. The beads were washed with saline immediately prior to their use to remove excess of calcium ions and unentrapped cells.

Ethanol production from cheese whey using immobilized Kluyveromyces marxianus NCDC 39 : The lactose and pH of

unclarified/clarified cheese whey was adjusted to 12% and 5.0, respectively. It was (50 ml in 250 ml capacity conical flask) then sterilized and inoculated with beads loaded to a known concentration of yeast cells. The flasks were incubated at 30^0C for 60 h (unless otherwise mentioned), under stationary conditions. The samples were analysed for change in pH, lactose and ethanol content.

Optimization studies : Both clarified and unclarified cheese whey were screened for their ethanol production potentials using immobilized *Kluyveromyces marxianus* NCDC 39. Cell load of 3.0 X 10^8 - 2.0 X 10^9 cells/50 ml of 48 h old culture was applied to determine the effect of cell density on ethanol production. Various incubation periods (48 - 96 h) were investigated to study the effect of time course on ethanol production. The initial adjustment of pH (3.0 - 7.0) was made with 1.0 N citric acid/1.0 N sodium hydroxide to study the influence of pH on ethanol production. To investigate the influence of agitation on ethanol production using immobilized yeast culture, the whey samples were incubated under stationary conditions in a BOD incubator and shaking conditions at 100 rpm on a rotary shaker (Certomat M, Germany). To study the effect of ethanol producing ability of immobilized culture, the alginate beads containing entrapped yeast cells were reused in clarified cheese whey at 72 h intervals. The fermented broth containing alginate entrapped yeast cells were filtered through a stainless steel filter under aseptic conditions at 72 h intervals. The alginate entrapped beads were washed with sterilized saline solution and then reused in the sterilized cheese whey for ethanol production.

Analytical methods

Total solids : The procedure of Indian Standards Institution (ISI 1977) was employed to calculate the contents of total solids in whey samples.

Titratable acidity : The method of AOAC (1975) was followed to estimate the acidity of whey samples.

Biochemical Oxygen Demand (BOD_5) : The biochemical oxygen demand was determined by the method of APHA (1989).

Chemical Oxygen Demand (COD) : The chemical oxygen demand was determined by closed reflux method (APHA 1989) using Hach COD reactor and Hach DR - 2000 spectrophotometer system (Hatch Co, USA).

Estimation of lactose : The lactose content was estimated according to the method of Perry and Doan (1950).

Estimation of tyrosine : The tyrosine value of whey was estimated by the method of Lowry et al (1951).

Estimation of ethanol concentration : The ethanol concentration in the fermented broth was estimated by the method of Holdeman et al (1977) using Microprocessor-based Nucon gas chromatograph (GC) equipped with flame ionization detector (F.I.D.) and a porapack column. The injector, detector and oven temperature of gas chromatograph were maintained at 200, 210 and 180^0C, respectively.

The samples were centrifuged at 5000 rpm in a refrigerated centrifuge (Beckman, Avanti™ 30, USA) at 4^0C for 10 min. The samples were injected in the injector of gas chromatograph with a microlitre syringe (Hemilton, USA) and observations were recorded. To calculate the ethanol content, the observations made in the unknown samples were compared with 1% (v/v) standard ethanol.

Fermentation efficiency and ethanol yield : Fermentation efficiency and ethanol yield ($Y_{p/s}$) of *Kluyveromyces marxianus* NCDC 39 were calculated as under :

$$\text{Fermentation efficiency } \% = \frac{\text{Actual ethanol recovery} \times 100}{\text{Theoretical ethanol production}}$$

$$\text{Theoretical ethanol production} = \text{Sugar utilization} \times 0.64$$

$$\text{Ethanol yield } (Y_{p/s}) = \frac{\text{Ethanol production, g}}{\text{Substrate utilized, g}}$$

Values expressed are average of three independent experiments.

Results and Discussion

Characteristics of cheese whey : Cheese whey was procured from Verka Milk Plant, Patiala, India and analysed for its physico-chemical characteristics. The results of physico-chemical analysis are presented in Table 1. Whey was greenish-yellow and sticky. The stickiness may be attributed to the presence of proteins and fats in whey. The values of acidity of different samples did not match with the pH of the sample. This was due to the fact that to maintain pH during processing of cheese in the milk plant, suitable salts were added. This may change the pH of the whey sample, whereas percent acidity of the sample was in terms of lactic acid present in the sample. The amounts of total solids, acidity, biochemical oxygen demand and chemical oxygen demand of the tested samples were much higher than the federal permissible limits governing the discharge of industrial effluents in India.

Screening of cheese whey for ethanol production using immobilized Kluyveromyces marxianus NCDC 39 : Both clarified and unclarified cheese whey was screened for ethanol production potentials using immobilized *Kluyveromyces marxianus.* A comparison of the results (Table 2) showed that ethanol production and lactose utilization were more in case of clarified cheese whey than in unclarified cheese whey. The ethanol production and lactose utilization in case of clarified cheese whey were 2.73% (v/v) and 76.40% (w/v), respectively. The corresponding values for ethanol production and lactose utilization for unclarified whey were 1.70% and 69.90%, respectively. After fermentation, a decrease in pH has been observed in both clarified and unclarified whey. The presence of proteins in the unclarified cheese whey hinders the fermentation efficiency of the yeast culture to some extent. The maximum yield of ethanol and lactose utilization has also been reported in clarified *paneer* whey in shake flask trials (Capoor and Singh 1986). These results suggest that clarified whey

TABLE 1. CHARACTERISTICS OF CHEESE WHEY

Sample No.	pH	Acidity %	Total solids, %, w/v	Biochemical oxygen demand, mg/l	Chemical oxygen demand, mg/l	Lactose, %, w/v	Tyrosine value, %	Fat %, w/v
1.	4.7	0.25	8.6	46,000	71,000	4.60	0.74	0.50
2.	5.5	0.27	9.1	48,700	61,300	5.40	0.80	0.60
3.	5.2	0.25	8.2	40,575	57,600	5.20	0.91	0.55
4.	4.9	0.30	9.5	50,825	72,400	4.60	0.87	0.65
5.	5.7	0.28	8.9	44,600	55,800	5.00	0.71	0.48

is more suitable for ethanol production using yeast culture. Keeping the above observations in view, clarified whey was selected for further experimentation.

Ethanol production from clarified cheese whey using immobilized Kluyveromyces marxianus NCDC 39 with size of inoculum as a function : To study the effect of concentration of entrapped cells on ethanol production from clarified whey, different cell loads (3.0×10^8 - 2.0×10^9 cells) were immobilized. A progressive increase in both ethanol production and lactose utilization with the increasing concentration of the entrapped cell load was observed up to a biomass concentration of 9.0×10^8 cells (Table 3). Thereafter, a decline in ethanol production, but an increase in lactose utilization was recorded. The maximum ethanol production (2.70%, v/v) was observed in a concentration of 9.0×10^8 cells (Fig. 1). The corresponding lactose utilization at this cell concentration was 76.20% (w/v). The cell concentration of 9.0×10^8 cells, which supported maximum ethanol production, was considered optimal and was selected for further investigations.

Ethanol production from clarified cheese whey using immobilized Kluyveromyces marxianus NCDC 39 with time-course as a function : A cell concentration of 9.0×10^8 cells was employed to determine the effect of retention time (48 - 96 h) on ethanol production from clarified cheese whey. The results (Table 4) showed that there were increases in ethanol production and lactose utilization by entrapped yeast cells, upto 72 h incubation. Thereafter, little improvement in ethanol production was observed. However, considerable increase in lactose utilization was recorded. The ethanol production and lactose utilization observed after 72 h of incubation were 3.08% (v/v) and 85.20% (w/v), respectively (Fig. 2). The ethanol production of 3.13% (v/v) and lactose utilization 94.51% (w/v) were recorded after 96 h of incubation. A time period of 72 h has been reported efficient for ethanol production using yeast cultures in both clarified and unclarified *paneer* whey (Capoor and Singh 1986). Keeping the above observation in view, an incubation period of 72 h was selected for further studies.

Ethanol production from clarified cheese whey using immobilized Kluyveromyces marxianus NCDC 39 with pH as a function : The pH of clarified cheese whey was initially adjusted between 3.0 - 7.0, to evaluate its effect on the ethanol production by immobilized *Kluyveromyces marxianus*. The results (Table 5) showed that there were increases in ethanol production and lactose utilization by the entrapped cells up to pH 5.0. Thereafter, a decline in ethanol production and

TABLE 2. ETHANOL PRODUCTION FROM CLARIFIED AND UNCLARIFIED CHEESE WHEY* USING IMMOBILIZED *KLUYVEROMYCES MARXIANUS* NCDC 39.

Type of whey	pH after fermentation	Lactose utilization, %, w/v	Ethanol production, %, v/v	Fermentation efficiency, %	Ethanol yield g/g
Clarified	4.21	76.40	2.73	73.3	0.47
Unclarified	4.38	50.15	1.70	69.9	0.44

*The lactose and pH of cheese whey were adjusted to 12.00% (w/v) and pH 5.00, respectively.

Fermentation conditions : Age of inoculum : 48 h; Biomass : 8.75×10^8 yeast cells; bead size : 3.0 mm; Temperature : 30°C; Incubation : 60h; Condition : Stationary.

TABLE 3. ETHANOL PRODUCTION FROM CLARIFIED CHEESE WHEY* USING IMMOBILIZED *KLUYVEROMYCES MARXIANUS* NCDC 39 WITH SIZE OF INOCULUM AS A FUNCTION

Inoculum size, number of yeast cells	pH after fermentation	Lactose utilization, %, w/v	Ethanol production, %, v/v	Fermentation efficiency, %	Ethanol yield, g/g
3.0×10^8	4.30	71.15	1.78	51.47	0.328
7.0×10^8	4.25	72.23	2.33	66.42	0.425
9.0×10^8	4.23	76.20	2.70	72.94	0.466
1.0×10^9	4.15	81.96	2.26	56.68	0.362
2.0×10^9	4.10	84.93	1.95	47.38	0.302

*The lactose and pH of cheese whey were adjusted to 12.00% (w/v) and pH 5.00, respectively.

Fermentation conditions : Age of inoculum : 48 h; bead size : 3.0 mm; Temperature : 30°C; Incubation : 60h; Condition : Stationary.

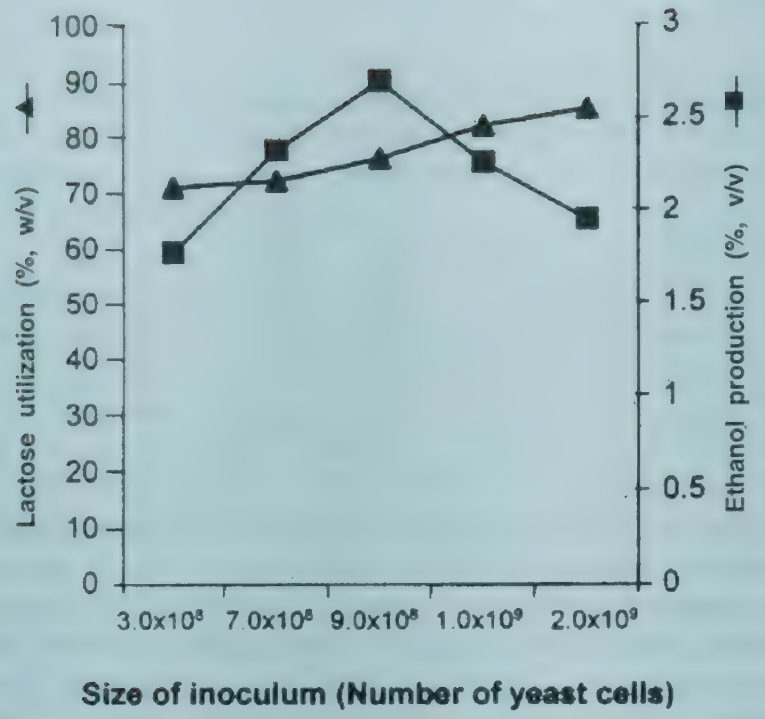

Fig. 1. Ethanol production from clarified cheese whey using immobilisezed *Kluyveromyces Marxianus* NCDC 39 with size of inoculum as a function.

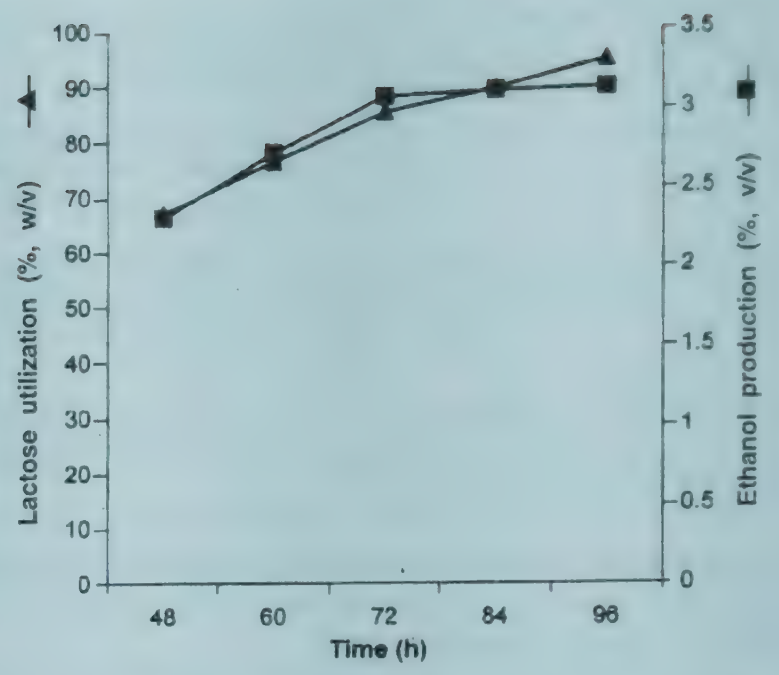

Fig. 2. Ethanol production from clarified cheese whey using immobilisezed *Kluyveromyces Marxianus* NCDC 39 with time-course as a function.

lactose utilization was observed. Ethanol production and lactose utilization at pH 5.0 were 3.14% (v/v) and 86.51% (w/v), respectively. The results showed the relationship between pH and ethanol production by the immobilized yeast cells. The optimum pH for ethanol production by immobilized yeast cells has been reported to be 5.5 (Marwaha and Kennedy 1984). Keeping the above observations in view, the pH 5.0 was considered optimal for ethanol production from cheese whey using *Kluyveromyces marxianus.*

Ethanol production from clarified whey using Kluyveromyces marxianus NCDC 39 with agitation as function : To determine the effect of agitation on ethanol production and lactose utilization by immobilized *Kluyveromyces marxianus,* the inoculated whey cultures were kept under stationary conditions in a BOD incubator and shaking conditions on a rotary shaker at 100 rpm. No marked difference in ethanol production and lactose utilization was observed under stationary and agitation conditions (Table 6). Therefore, stationary conditions were selected for further studies.

Recycling of alginate entrapped Kluyveromyces marxianus NCDC 39 : To test the suitability of *Kluyveromyces marxianus* to carry out long term ethanol production, the alginate beads carrying the entrapped yeast cells were recycled. After 72 h of incubation, the fermented whey was replaced with fresh whey. The results are presented in Table 7. The reuse of beads up to fifth cycle showed no marked change in their ability to carry out ethanol production and lactose utilization. But, thereafter, a decline in both lactose utilization and ethanol production was observed. The experiment was terminated

TABLE 4. ETHANOL PRODUCTION FROM CLARIFIED CHEESE WHEY* USING IMMOBILIZED *KLUYVEROMYCES MARXIANUS* NCDC 39 WITH TIME-COURSE AS A FUNCTION.

Time, h	pH after fermentation	Lactose utilization, %, w/v	Ethanol production, %, v/v	Fermentation efficiency, %	Ethanol yield, g/g
48	4.25	67.01	2.32	71.20	0.455
60	4.20	76.42	2.73	73.37	0.470
72	4.16	85.20	3.08	74.31	0.475
84	4.13	89.33	3.11	72.04	0.460
96	4.10	94.51	3.13	68.23	0.435

*The lactose and pH of cheese whey were adjusted to 12.00% (w/v) and pH 5.00 respectively.

Fermentation conditions : Age of inoculum : 48 h; Biomass : 9.0 X 10^8 yeast cells; bead size : 3.0 mm; Temperature : 30°C; Incubation : 60 h; Condition : Stationary.

TABLE 5. ETHANOL PRODUCTION FROM CLARIFIED CHEESE WHEY* USING IMMOBILIZED *KLUYVEROMYCES MARXIANUS* NCDC 39 WITH pH AS A FUNCTION.

Initial pH	pH after fermentation	Lactose utilization, %, w/v	Ethanol production, %, v/v	Fermentation efficiency, %	Ethanol yield, g/g
3.00	2.40	62.21	1.76	58.40	0.372
4.00	3.60	78.02	2.10	55.48	0.352
5.00	4.15	86.51	3.14	74.69	0.477
6.00	5.30	81.40	2.60	65.70	0.420
7.00	6.25	77.43	2.47	65.87	0.420

*The lactose of cheese whey was adjusted to 12.00% (w/v)

Fermentation conditions : Age of inoculum : 48 h; Biomass 9.0 X 10^8 yeast cells; bead size : 3.0 mm; Temperature : 30°C; Incubation : 72 h; Condition : Stationary.

TABLE 6. ETHANOL PRODUCTION FROM CLARIFIED CHEESE WHEY* USING IMMOBILIZED *KLUYVEROMYCES MARXIANUS* NCDC 39 WITH AGITATION AS A FUNCTION.

Condition	pH after fermentation	Lactose utilization, %, w/v	Ethanol production, %, v/v	Fermentation efficiency, %	Ethanol yield, g/g
Stationary	4.16	85.23	3.09	74.61	0.477
Agitation	4.10	91.51	3.15	70.65	0.451

*The lactose and pH of cheese whey were adjusted to 12.00% (w/v) and pH 5.00, respectively.

Fermentation conditions : Age of inoculum : 48 h; Biomass : 9.0×10^8 yeast cells; bead size : 3.0 mm; Temperature : 30^0C; Incubation : 72 h; Condition : Stationary.

TABLE 7. RECYCLING OF IMMOBILIZED YEAST CULTURE WITH LACTOSE UTILIZATION AND ETHANOL PRODUCTION AS A FUNCTION

Cycle number	pH after fermentation	Lactose utilization, %, w/v	Ethanol production, %, v/v	Fermentation efficiency, %	Ethanol yield, g/g
1	4.08	86.03	3.05	72.72	0.465
2	4.10	85.61	3.02	72.56	0.464
3	4.13	85.34	3.00	72.47	0.463
4	4.20	84.90	2.97	72.00	0.459
5	4.24	84.42	2.90	70.89	0.452
6	4.31	83.71	2.70	66.35	0.425
7	4.40	82.13	2.52	63.37	0.404
8	4.46	81.21	2.34	59.16	0.377

*The lactose and pH of cheese whey were adjusted to 12.00% (w/v) and pH 5.00, respectively.

Fermentation conditions : Age of inoculum : 48 h; Biomass : 8.75×10^8 yeast cells; bead size : 3.0 mm; Temperature : 30^0C; Incubation : 72 h; Condition : Stationary.

after 8th cycle, when the ethanol production was dropped to 2.34% (v/v). The decrease in ethanol production and lactose utilization after the repeated use of alginate entrapped yeast cells may be attributed to the degeneration of the culture and leakage of the cells from the matrix.

The above experimentation carried out in flask culture at bench scale will provide very useful guidelines for the process development and scale-up studies on ethanol production from cheese whey.

References

AOAC (1975) Official Methods of Analysis, 12th edn. Association of Official Analytical Chemists, Washington, DC USA

APHA (1989) Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC USA

Capoor AK, Singh K (1986) Protein and alcohol production by yeast in clarified and supplemented whey. Ind J Dairy Sci 39 : 54-59

Fritzscha B (1986) Soil geological and hydrogeological problem and the disposal of dairy product waste water. Zeitschrift fuer Angewandte Geologie 32 : 131-134

Gandhi DN (1984) Development of soft whey drink - Acidowhey. Dairy Guide. 6 : 41-43

Holdeman LV, Cato EP, Moore WEC (1977) Anaerobe Laboratory Manual, 4th edn. Virginia Polytechnic Institute, Blacksburg, Virginia, USA

Indian Standards Institution (1977) Methods of Sampling and Tests for Industrial Effluents. Part - I, Indian Standards Institution, New Delhi

Kosikowski FV (1978) Cheese and Fermented Milk Foods. Edward Brothers Inc, Ann Arbor Michigan, USA. p. 668

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent, J Biol Chem 193 : 265-275

Marwaha SS, Kennedy JF (1984) Ethanol production from whey permeate by immobilized yeast cells. Enz Microb Technol 6 : 18-21

Marwaha SS, Kennedy JF (1988) Whey - pollution problem and potential utilization. Int J Food Sci Technol 23 : 323-336

Marwaha SS, Kennedy JF, Tewari HK, Redu A (1988) Development of non-conventional technology employing immobilized yeast cells for the treatment of dairy industry effluents. Int Ind Biotechnol 8 : 17-23

Marwaha SS, Sethi RP (1984) Utilization of dairy waste for vitamin B_{12} fermentation. Agric Wastes 9 : 111-130

Navarro JM, Burand G, Duterurtre B, Moll M, Corrieu G (1976) Whey wine from concentrates of reconstituted acid whey powder, Incd Alim. Agric. 93 : 965-970

Perry NA, Doan FJ (1950) A picric acid method for the simultaneous determination of lactose and sucrose in dairy products. J Dairy Sci 33 : 176-185

Robinson BP, Short JL, Marhsall KR (1976) Traditional lactabumin - manufacture, properties and uses. NZ J Dairy Sci Technol 11 : 114-126

Tiwana NS (1985) Nature and magnitude of water pollution in Punjab. In : Proceedings of Seminar on Status of Environment Pollution, PAU, Ludhiana, India. pp 1-7

Wheatland AB (1974) Treatment of waste water from dairies and dairy product factories - methods and systems. J Soc Dairy Technol 27 : 71-79

Zall RR (1984) Trends in whey fractionation and utilization - A global perspective. J Dairy Sci 67 : 2621-2629

Received 8 July 2000; revised 2 July 2001; accepted 9 December 2001

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 26-32

Effect of Food Preservatives on *Acinetobacter* genospecies Isolated from Meat

S.C. SAHA AND B.A. CHOPADE*

Department of Microbiology,
University of Pune, Ganeshkhind, Pune 411 007, India

Six genospecies of *Acinetobacter* such as *Acinetobacter calcoaceticus, A. lwoffii, A. baumannii, A. johnsonii, A. junii and A. haemolyticus* were isolated from fresh, spoiled, refrigerated (4^{0}C) and freezer (-20^{0}C) meat. All *Acinetobacter* were tested against different food preservatives used commercially. Of 28 strains, 13 showed tolerance to 0.2 - 0.3% sodium benzoate and the remaining 15 were sensitive at these concentrations, where no growth was found at 0.5%. Eighteen genospecies were sensitive to 0.1-0.2% $NaNO_2$, while 6 strains showed tolerance to 0.3%. Sixteen strains of all six genospecies grew at 0.1 - 0.2% potassium sorbate and growth was totally inhibited at 0.5%. Eighteen strains of *Acinetobacter* genospecies showed tolerance of salt (NaCl) up to 5%; except *A. haemolyticus* (M27) which was sensitive to 1%, whereas a strain of *A. baumannii* (M25) showed tolerance to 10%. No growth was found in citric acid, propionic acid and malonic acid at 0.2 to 0.5%. Twenty three strains showed the tolerance to lactic acid at 0.05 to 0.1%, whereas these strains showed tolerance to acetic acid at 0.1 - 0.2%. The time-kill curves of six *Acinetobacter* genospecies were made at room temperature (30^{0}C) and refrigerator temperature (4^{0}C). The combination of sodium nitrite and sodium chloride was found to be more effective against *Acinetobacter* genospecies as compared to a single preservative. Eighteen strains of *Acinetobacter* genospecies were found to survive at 0, 2, 6 & 12 h and even 4 strains of genospecies *A. calcoaceticus* and 5 strains of *A. baumannii* grew in 24 h at room temperature (30^{0}C) with sodium benzoate, benzoic acid and citric acid. None of *Acinetobacter* genospecies survived up to 24 h at 4^{0}C with preservatives.

Keywords : *Acinetobacter,* Genospecies, Preservatives, Refrigerator temperature, Room temperature, Time-kill curve.

Microbial contamination in meat is a major concern, causing spoilage of meat and consequent economic loss and public health problems. Microbial safety and shelf-life of meat depend on minimizing the initial level of microbial contamination, preventing or limiting the rate of microbial growth, or destroying microbial populations. The type of bacterial flora involved has been studied by various workers (Vijaya Rao et al. 1983; Gupta et al. 1983; Narasimha Rao et al. 1998). The prevention of spoilage by inhibiting or destroying the microorganisms is the basis of food preservation. This can be done by chemical treatment, curing, dehydration or thermal processing (Surekha and Reddy 1999). Compounds such as salt, vinegar, organic fruit acids (natural forms), wood smoke and various spices used for the preservation of food for centuries are regarded as traditional preservatives. Some of the other permitted preservatives such as benzoic acid, sorbic acid and its salts, propionic acid and malonic acid are used for the preservation of meat and meat products at different concentrations (Jensen 1954; Russel et al. 1982; Cook and Pierson 1983; Gill and Penny 1985; Sankaran et al. 1986; Acuff et al. 1987; Surekha and Reddy 1999). It should be noted that a single preservative is not active against all spoilage microorganisms. Attempts have been made to compensate for this by combining various preservatives-mainly weak acids with salt (NaCl) combinations (Surekha and Reddy 1999). Depending upon the type of preservative used, the final state at which the microorganisms are killed is reached within a few days or weeks, at the usual applied concentrations (MIC or SIC).

The psychrotrophic genera of *Acinetobacter* and *Moraxella* have been reported by many investigators to be a part of the normal flora and the important spoilage organism of fresh meats (Dainty et al. 1983). *Acinetobacter* and *Moraxella* constitute important group of microorganisms though *Pseudomonas* is the predominant genus of bacteria in fresh meats allowed to spoil aerobically at refrigerator temperatures (Ayres 1960 a, b; Gill and Newton 1980; Eribo and Jay 1985; Narashima Rao and Sreenivasamurthy 1985; Gennari et al. 1989; Saha and Chopade 2001). *Acinetobacter* plays an important role in the spoilage of meat and foods under various conditions (Jay and Shelf 1978; Banwart 1981; Choudhari et al. 1990; Jay 1991; Frazier and Westhoff 1995). In recent years, *Acinetobacter* has emerged as an important nosocomial pathogen and it is one of the most resistant bacteria to antibiotics and metals (Deshpande et al. 1993; Chopade et al. 1994; Dhakephalkar and Chopade 1994; Deshpande and Chopade 1994; Shakibaie et al. 1999). Though *Acinetobacter* spp. has been isolated by many investigators from food but at the best of our knowledge, there is no report on the effect of preservatives on food from *Acinetobacter.*

The main objective of the present investigation was to study the effect of preservatives in combination on the shelf-life and safety attributes of meat.

Materials and Methods

Microorganisms used : Six genospecies of *Acinetobacter* such as - *Acinetobacter calcoaceticus, A. lwoffii, A. baumannii, A. johnsonii, A. junii and A. haemolyticus* were used as test organisms for the determination of minimal inhibitory concentration (MIC) and sub-inhibitory concentration of preservatives (SIC) and for the time-kill curves with preservatives. These experiments were performed in the months of June and July, 2000. At this time, the average room temperature was approximately 30 - 32^{0}C. All organisms were maintained on CLED agar slants and plates at 4^{0}C.

* *Corresponding Author E-mail : chopade@unipune.ernet.in*

Source of the organisms : Acinetobacter spp. were isolated, characterized and identified from fresh meat, spoiled meat, refrigerator meat (4^{0}C) and freezer meat (-20^{0}C) (Baumann 1968; Juni 1972; Holton 1983; Eribo and Jay 1985; Bouvet and Grimont 1986, 1987; Choudhari et al. 1990; Gennari et al 1992; Saha and Chopade 2001).

Preservatives used : The following preservatives were used for MIC and time-kill-curve studies : Sorbic acid (LOBA Chemie, Mumbai), Benzoic acid (s. d. fine chemie, Mumbai), Lactic acid (E-MERCK, Mumbai, Acetic acid (SRL, Mumbai), Citric acid (SRL, Mumbai), Propionic acid (SRL, Mumbai), Malonic acid (E-MERCK, Mumbai), Sodium benzoate (LOBA, Chemie, Mumbai), Potassium sorbate (LOBA Chemie, Mumbai), Sodium nitrite (Qualigens, Mumbai) and Sodium chloride (HiMedia, Mumbai). All preservatives were of analytical grade. A fresh solution of each preservative was used in all experiments.

Determination of minimal inhibitory concentration (MIC) for Acinetobacter *spp. to preservatives : Acinetobacters* were grown in Nutrient broth at 30^{0}C for overnight at pH 6.8. Number of cells in per ml of overnight broth cultures were determined as per the standard method of McFarland Scale (Gerhardt et al. 1994). Preservatives were prepared at different concentrations in sterile distilled water and filter sterilised (0.22 μm pore size cellulose acetate membrane filter, Sartorius, Germany). Different concentrations of preservatives were added into sterile Nutrient agar medium (25 ml / plate) at 50 to 55^{0}C and poured into 80 mm petri plates. Plates were inoculated by spot inoculation technique with all *Acinetobacter* genospecies (2-4 μl/spot) from known count of overnight grown culture and incubated at 30^{0}C for 24 h. Growth was observed for 24 to 48 h. The minimal inhibitory concentration (MIC) was the concentration at which no visible growth of organism was observed. Sub-inhibitory concentrations (SIC) were determined from the MIC of the organisms.

The time-kill curves of Acinetobacter *genospecies with preservatives :* Sterile Nutrient broth tubes containing different preservatives in SIC and MIC range (%) were inoculated with one ml of inoculum of each culture of known bacterial count (x10^6) and incubated at 30^0 and 4^{0}C for 24 h. Total counts were made at different intervals (0, 2, 6, 12, and 24 h) followed by micro-dilution technique (Krogstad and Moellering 1986) and expressed as log 10 of colony forming units (cfu/ml). The pH was determined as per the procedure described in AOAC (1984) using glass electrodes. Each experiment was repeated for three times at both the temperatures (30^{0}C and 4^{0}C). For plotting graph - the-time kill curves, Microsoft Excel programme was used (Walkenbach 1995).

Results and Discussion

Minimum concentrations for inhibition of the *Acinetobacter lwoffii, A. johnsonii, A. baumannii, A. calcoaceticus, A. junii and A. haemolyticus* were determined at pH 4.5. Seven weak acid preservatives and five salt preservatives were used as meat preservatives (Table 1). In weak acid preservatives MIC values varied from 0.1% for sorbic acid found in *A. lwoffii, A. junii and A. haemolyticus,* to 0.3% for acetic acid in *A. johnsonii, A. baumannii* and *A. calcoaceticus* (Table 1). In the different salt preservatives, the MIC values varied from 0.1% for sodium nitrite against *A. haemolyticus* to 6-10% for sodium chloride against *A. baumannii* (Table 1).

Minimal inhibitory concentrations (MIC) for sodium nitrite were 0.1% against *A. haemolyticus,* whereas it is 0.2% against *A. lwoffii, A. johnsonii,* as well as *A. junii.* and 0.3% against *A. baumannii* and *A. calcoaceticus.* The MIC values in combination of sodium chloride + sodium nitrite were 0.8 ± 0.05% against *A. haemolyticus,* 3.5 ± 0.1% against *A. lwoffii, A. johnsonii,* and *A. junii.* whereas it is 4 ± 0.2% against *A. calcoaceticus* and is 5 ± 0.2% against *A. baumannii* (Table 1). Similar results have been reported by Russel et al (1982); Stratford and Anslow (1998); Lambert and Stratford (1998); Ogbadu (1999). These authors worked on the food spoilage as well as pathogenic organisms. The organisms were mainly Gram negative bacteria and also few species of Gram positive bacteria and yeast. Thomas (1999) reported that the use of sorbate in meat in USA and UK is restricted to the surface

TABLE 1. MINIMAL INHIBITORY CONCENTRATION (MIC) OF *ACINETOBACTER* SPP. FOR FOOD PRESERVATIVES.

Name of bacteria	Minimal-Inhibitory concentration of food preservatives* at %											
	SA	BA	LA	PA	MA	AA	CA	Na-Bt.	K-Srt	$NaNO_2$	NaCl	NaCl+Na-NO_2
A. lwoffii	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.4	0.2	0.2	5	3.5 + 0.1
A. johnsonii	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.4	0.4	0.2	6	3.5 + 0.1
A. baumannii	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.5	0.5	0.3	6-10	5 + 0.2
A. calcoaceticus	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.5	0.5	0.3	6-7	4 + 0.2
A. junii	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.2	4	3.5 + 0.1
A. haemolyticus	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.3	0.2	0.1	1	0.8 + .05

*SA = Sorbic acid; BA = Benzoic acid; LA = Lactic acid; PA = Propionic acid; MA = Malonic acid;

AA = Acetic acid; CA = Citric acid; NaBt. = Sodium Benzoate; K-Srt. = Potassium Sorbate.

treatment of dried products up to 1000 to 3000 ppm. Maximum permitted levels of nitrites in cured meat in UK and Canada is 200 mg/kg (Singhal and Kulkarni 1999). The combination of sodium chloride and sodium nitrite was found to be as one of the effective preservatives for meat preservation (Table 1). This finding is in accordance with the reports of Russel et al. (1982); Surekha and Reddy (1999); Singhal and Kulkarni (1999). These investigators showed that the combination of 3.5% salt + 200 ppm nitrite was the most effective preservative for the preservation of cured meat and meat products like bacon, bolonga, frankfurters, corned beef, ham, sausages etc. Similarly, Ziauddin et al (1993) also found that different levels of lactic acid in combination with 0.5% sodium chloride were more effective in inhibiting growth of both the spoilage and pathogenic bacteria in fresh meat.

The sub-inhibitory concentrations of *Acinetobacter* spp. were determined according to the same procedure followed for the minimal concentration for inhibition and the results are shown in Table 2. The sub-inhibitory concentration was just the lower value of the minimal inhibitory concentration. The sub-inhibitory concentrations of weak acid preservatives was 0.05% for sorbic acid against *A. lwoffii, A. junii* and *A. haemolyticus,* 0.2% for acetic acid against *A. johnsonii, A. baumannii* and *A. calcoaceticus,* while it was 0.1% for acetic acid against *A. junii* and *A. haemolyticus* (Table 2). The sub-inhibitory concentration for sodium nitrite was 0.05% against *A. haemolyticus,* 0.1% against *A. lwoffii, A. johnsonii* and *A. junii,* 0.2% against *A. baumannii* and *A.calcoaceticus.* For potassium sorbate, it was 0.1% against *A. lwoffii* and *A. haemolyticus,* 0.2% is against *A. johnsonii* and *A. junii.* While it was 0.3% against *A. baumannii* and *A. calcoaceticus.* SIC for sodium benzoate was 0.2% against *A. lwoffii, A. johnsonii. A. junii* and *A. haemolyticus.* 0.3% was found against *A. baumannii* and *A. calcoaceticus.* SIC values for sodium chloride ranged from 0.5% against *A. haemolyticus* to 6% against *A. baumannii.* The range of SIC values in combination for sodium chloride + sodium nitrite varied from 0.5 ± 0.02% against *A. haemolyticus* and 3.5 ± 0.1% against *A. baumannii* and *A. calcoaceticus* (Table 2). These results indicate that *A. haemolyticus* and *A. junii* were the more sensitive to food preservatives than other genospecies of *Acinetobacter.*

Killing rate of *Acinetobacter* genospecies when grown on CLED agar plates in different time intervals (0, 2, 6, 12 & 12h) with sub-inhibitory concentration (SIC) levels of different weak acid and salt preservatives are shown in Fig. 1 to 6.1 at 30^{0}C and 4^{0}C, respectively. Fig. 1 indicates the time-kill curve of *A. lwoffii* at 30^{0}C, where growth was found to be inhibited in-between 6 and 12 h interval against all preservatives except sodium benzoate, whereas the organism survived up to 12 h and growth was found to be inhibited in between 12 and 24 h (Fig. 1). At 4^{0}C, the organism was found to be killed within 2 to 6 h against lactic acid, sorbic acid and sodium nitrite, whereas the organism was found to be killed at this temperature within 12 h against citric acid, benzoic acid and sodium benzoate (Fig. 1.1). Growth of *A. johnsonii* was found to be inhibited within 12 to 24 h of incubation at 30^{0}C against all preservatives (Fig. 2), whereas the organism was found to be killed after 6 h incubation against lactic acid, sorbic acid and sodium nitrite at 4^{0}C but exceptions were in citric acid, benzoic acid and sodium benzoate (12 h) (Fig. 2.1). Growth of *A. baumannii* was found to be inhibited at 24 h against all preservatives at 30^{0}C (Fig. 3) whereas no growth was found after 12 h incubation at 4^{0}C (Fig. 3.1). *A calcoaceticus* was found to be inhibited within 12 to 24 h against all preservatives at 30^{0}C (Fig. 4), whereas it was found to be killed within 6 to 12 h interval at 4^{0}C (Fig. 4.1). The growth of *A. junii* was found to be inhibited within 6 h at 30^{0}C against all the preservatives (Fig. 5) whereas, no growth was found after 2 h at 4^{0}C (Fig. 5.1). Growth of *Acinetobacter haemolyticus* was found to be inhibited within 4 to 6 h against all preservatives, exception being in sodium benzoate (12 h) at 30^{0}C (Fig. 6). whereas no growth was found after 2 h at 4^{0}C exception being

TABLE 2. SUB-INHIBITORY CONCENTRATION (SIC) OF *ACINETOBACTER* SPP. FOR FOOD PRESERVATIVES

Name of bacteria	Sub-Inhibitory concentration of food preservatives* (SIC) at %											
	SA	BA	LA	PA	MA	AA	CA	Na-Bt.	K-Srt	Na NO_2	NaCl	NaCl+Na $-NO_2$
A. lwoffii	0.05	0.1	0.05	0.1	0.1	0.1	0.1	0.2	0.1	0.1	4	3.5 + 0.05
A. johnsonii	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1	5	3.5 + 0.05
A. baumannii	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.3	0.3	0.2	5-6	3.5 + 0.1
A. calcoaceticus	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.3	0.3	0.2	5	3.5 + 0.1
A. junii	0.05	0.1	0.05	0.1	0.1	0.1	0.1	0.2	0.2	0.1	3	2.5 + 0.05
A. haemolyticus	0.05	0.1	0.05	0.1	0.1	0.1	0.1	0.2	0.1	0.05	0.5	0.5 + 0.02

*SA = Sorbic acid; BA = Benzoic acid; LA = Lactic acid; PA = Propionic acid; MA = Malonic acid,

AA = Acetic acid; CA = Citric acid; NaBt. = Sodium Benzoate; K-Srt. = Potassium Sorbate

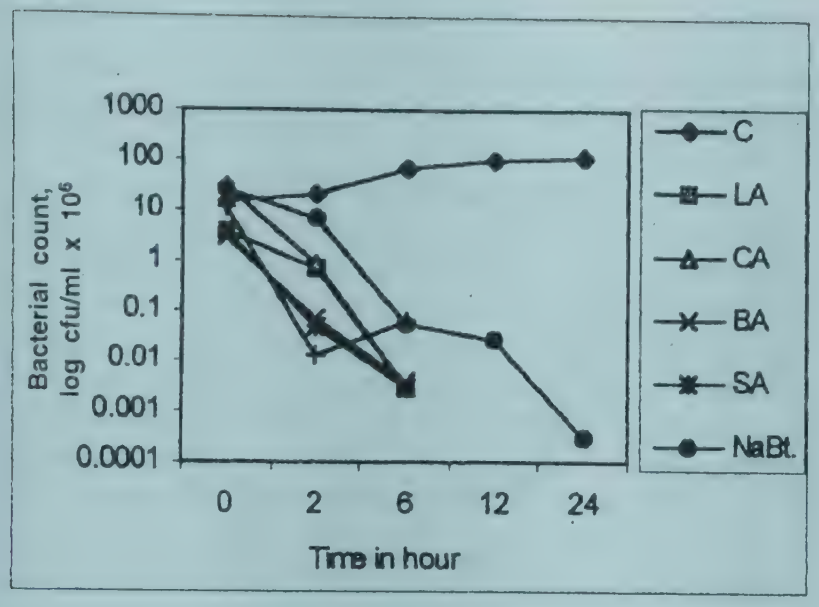

Fig. 1. Time-kill curve of *A. lwoffii* to preservatives at room temperature (30^{0}C)

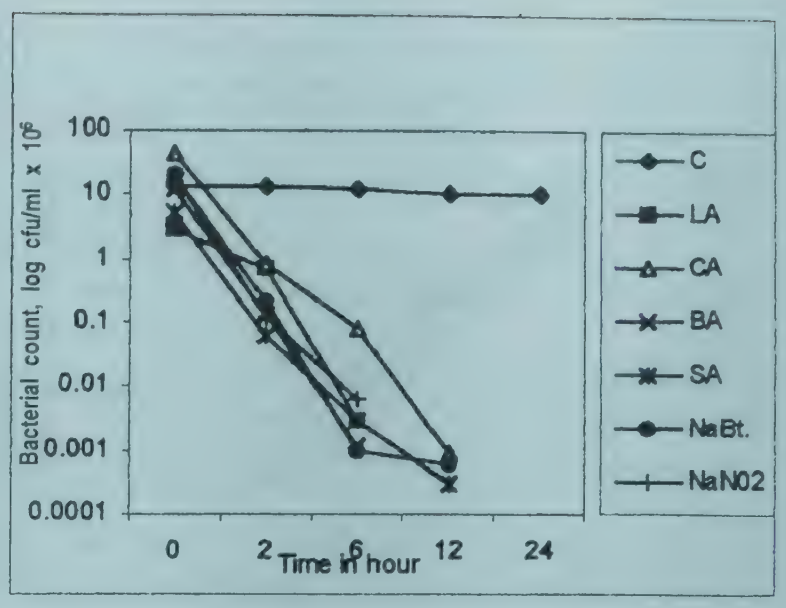

Fig. 2.1 Time-kill curve of *A. johnsonii* to preservatives at 4^{0}C

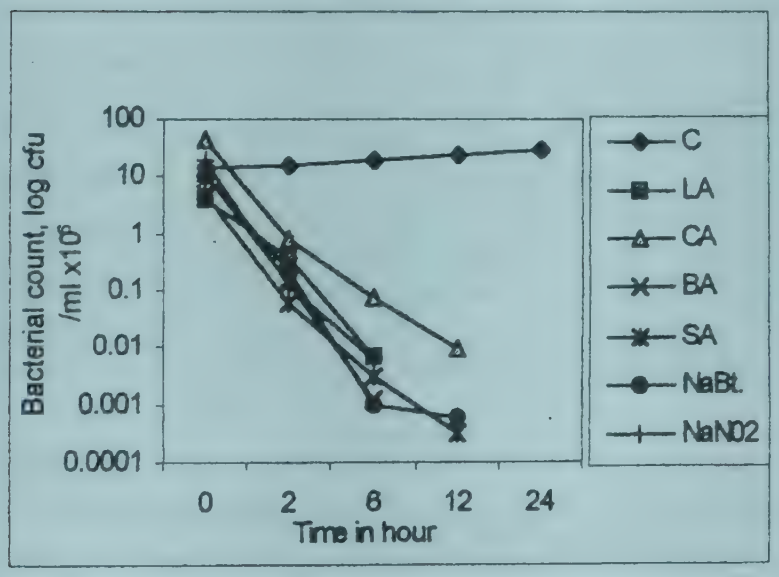

Fig. 1.1 Time-kill curve of *A. lwoffii* to preservatives at 4^{0}C

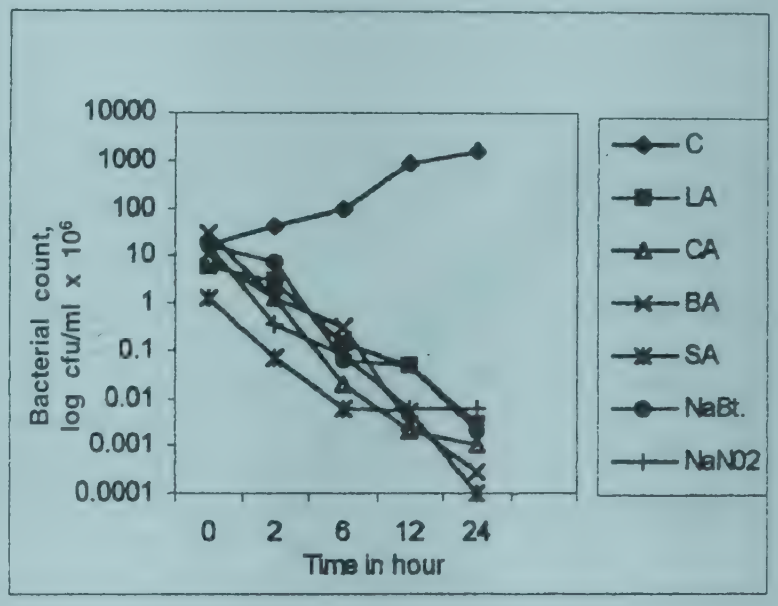

Fig. 3. Time-kill curve of *A. baumannii* to preservatives at room temperature (30^{0}C)

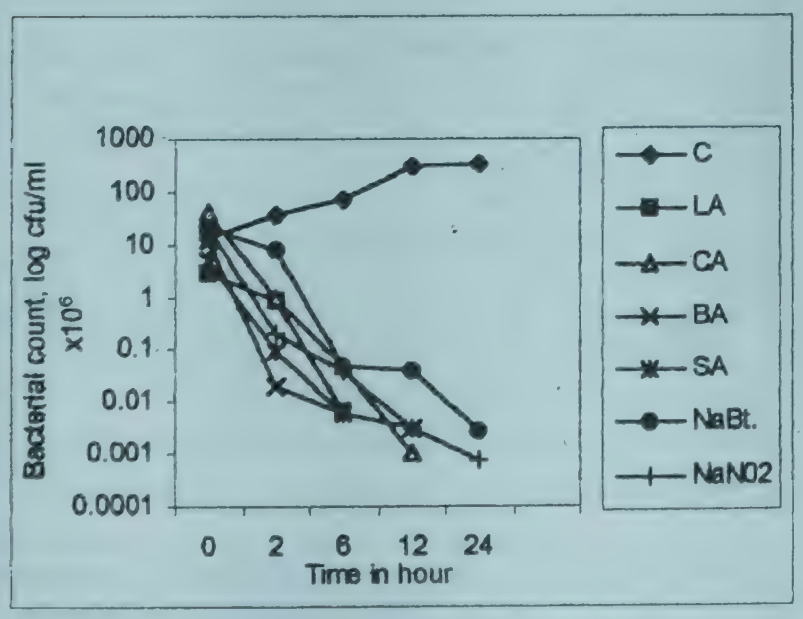

Fig. 2. Time-kill curve of *A. johnsonii* to preservatives at room temperature (30^{0}C)

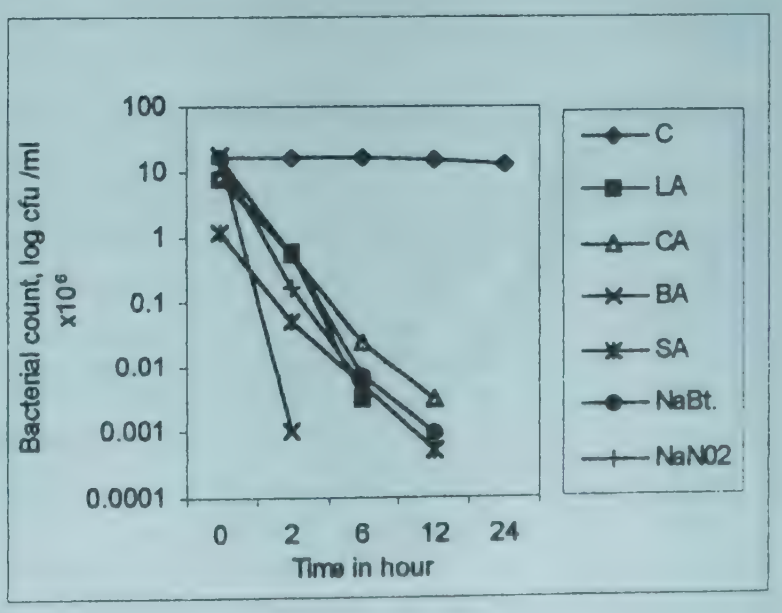

Fig. 3.1 Time-kill curve of *A. baumannii* to preservatives at 4^{0}C

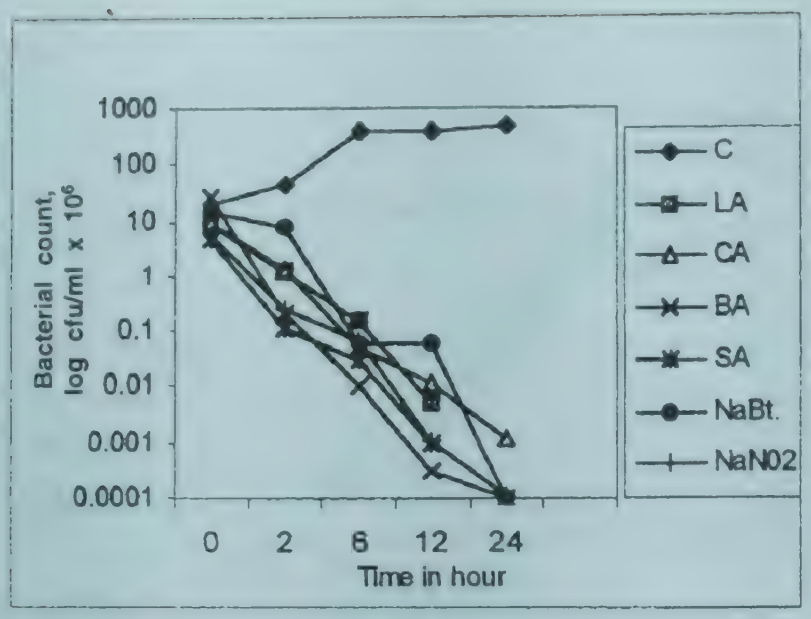

Fig. 4. Time-kill curve of *A. calcoaceticus* to preservatives at room temperature (30°C)

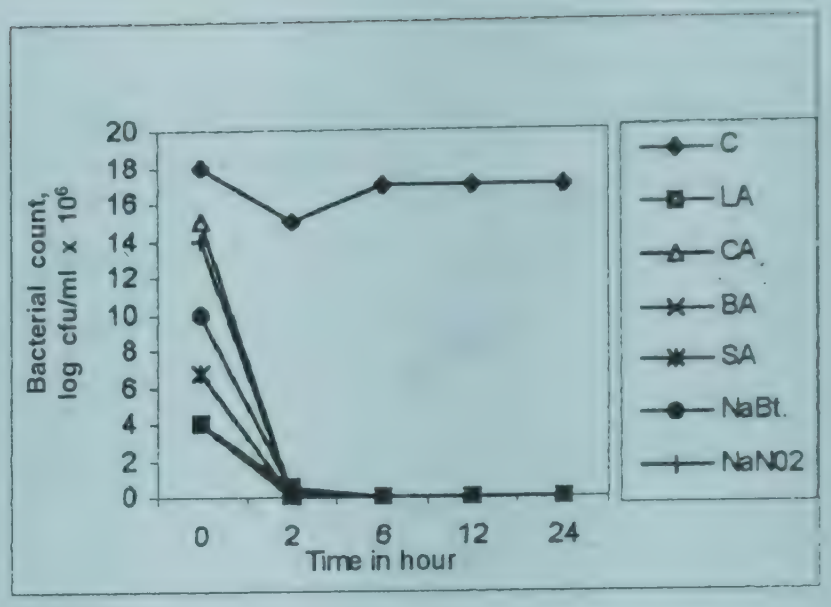

Fig. 5.1 Time-kill curve of *A. junii* to preservatives at 4°C

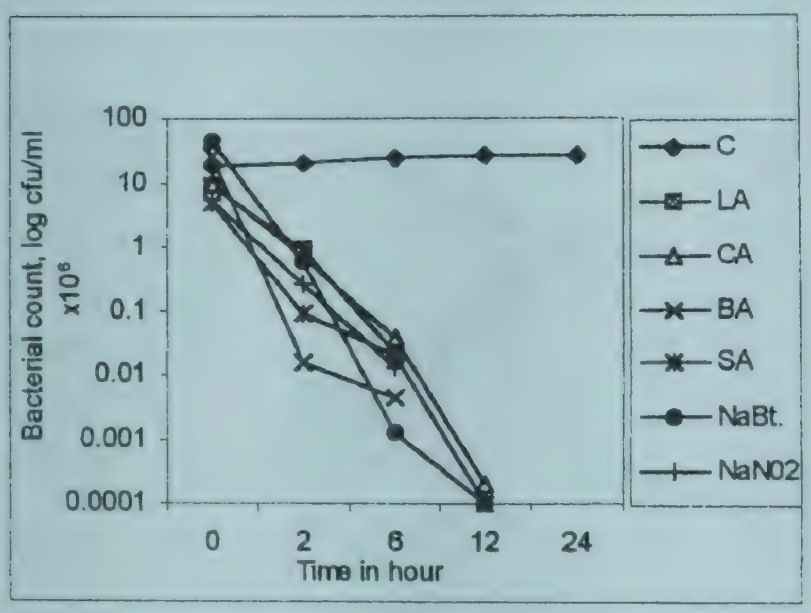

Fig. 4.1 Time-kill curve of *A. calcoaceticus* to preservatives at 4°C

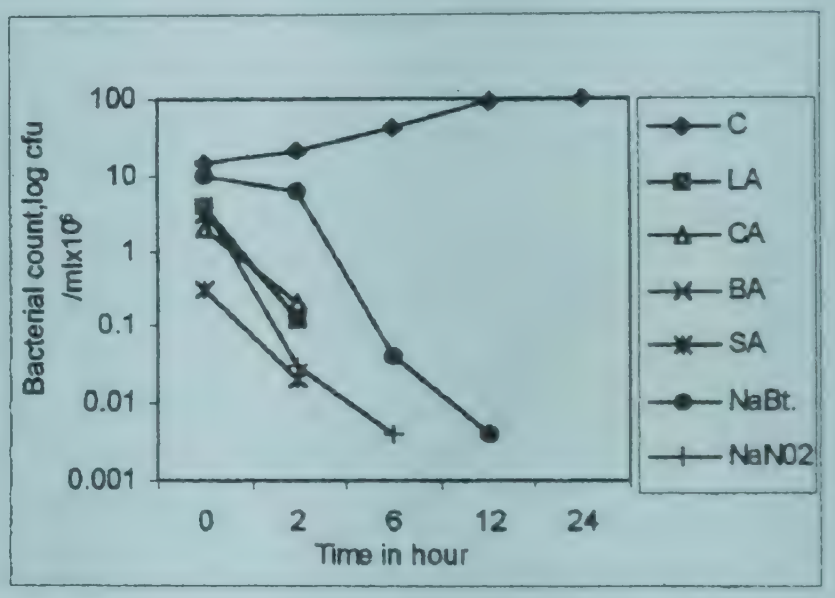

Fig. 6. Time-kill curve of *A. haemolyticus* to preservatives at room temperature (30°C)

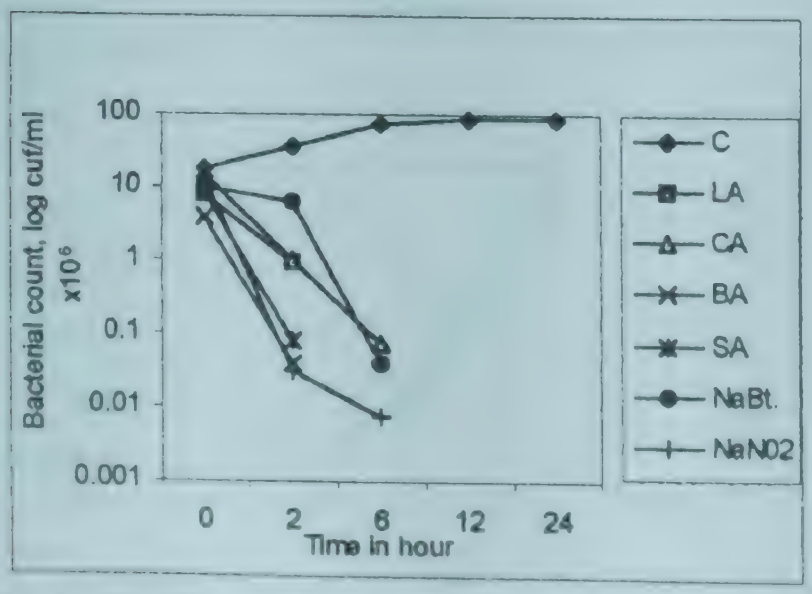

Fig. 5. Time-kill curve of *A. junii* to preservatives at room temperature (30°C)

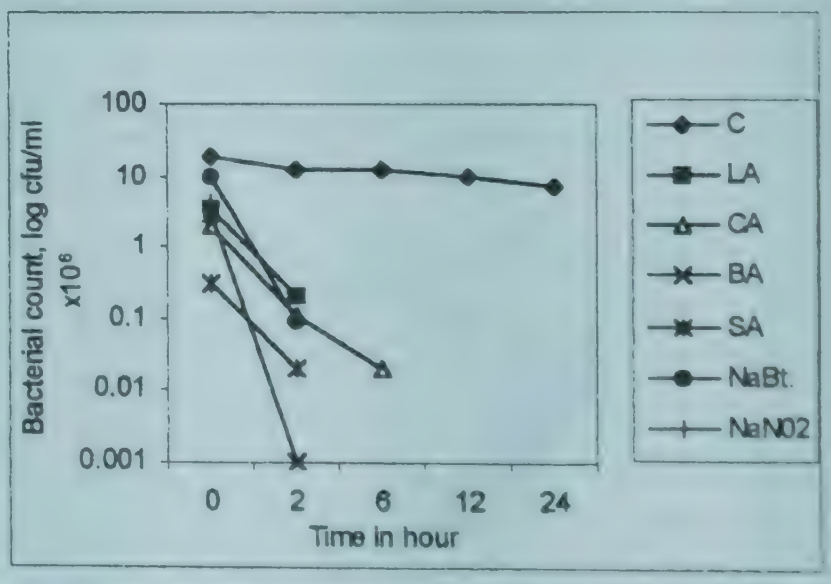

Fig. 6.1 Time-kill curve of *A. haemolyticus* to preservatives at 4°C

C = Control; LA = Lactic acid; CA = Citric Acid; BA = Benzoic acid; SA = Sorbic acid; NaBt. = Sodium benzoate; and $NaNO_2$ = Sodium nitrite

Fig. 4 and 4.1 to 6 and 6.1 - Effect of different food preservatives on *Acinetobacter* genospecies at room temperature (30°C) and at low temperature (4°C)

in sodium benzoate (8 h) (Fig. 6.1).

These findings indicated that *Acinetobacter junii* and *A. haemolyticus* were much more sensitive to all preservatives at room temperature (30^0C) and their killing rate was higher at low temperature (4^0C) as compared to other genospecies of *Acinetobacter*. These results also showed that the tolerance of preservatives was higher to *A. baumannii* and *A. calcoaceticus* among other genospecies of *Acinetobacter* and their killing rate were also lower in both at low temperature (4^0C) and room temperature (30^0C). The growth of most of the *Acinetobacter* genospecies were found to be at logarithmic stages up to 24 h at room temperature (30^0C), whereas growth was found to be at stationary phases after 24 h (except *A. lwoffii* and *A. haemolyticus*). These findings agreed with the findings of Bouvet and Grimont (1986) with respect to the growth of *Acinetobacter* genospecies in different temperatures.

Among the preservatives, sorbic acid, lactic acid, benzoic acid, sodium nitrite and the combination of sodium chloride + sodium nitrite were found to be the much more effective preservatives against *Acinetobacter* genospecies. These findings are in accordance with those of Ziauddin et al (1993); Stratford and Anslow (1998); Lambert and Stratford (1998); Thomas (1999); Singhal and Kulkarni (1999). Ziauddin (1993) showed that the combination of lactic acid + sodium chloride was more effective than a single lactic acid as preservative. Stratford and Anslow (1998); and Thomas (1999) found that the effect of sorbic acid on yeast is higher as compare to other weak acid preservatives. Singhal and Kulkarni (1999) observed that the combination of sodium nitrite and sodium chloride was more effective on spoilage organisms than a single preservative sodium nitrite.

Conclusions

It may be concluded that *Acinetobacter* genospecies can be much more sensitive to different weak acid preservatives and salt preservatives at low temperature (4^0C) as compared to room temperature (30^0C). The time-kill rate of *Acinetobacter haemolyticus* and *A. junii* is higher at low temperature (4^0) as compared to other genospecies with all preservatives. Sorbic acid, lactic acid, sodium nitrite ($NaNO_2$) and the combination of sodium nitrite and sodium chloride are the more effective preservatives against *Acinetobacter* genospecies among all other preservatives used.

Acknowledgements

The first author is thankful to Indian Council for Cultural Relations (ICCR), Ministry of Human Resource Development (HRD), Government of India, New Delhi for providing the scholarship to carry out this doctoral research work and also is very much grateful to Ministry of Education, Government of Bangladesh for granting the deputation for during this research in India.

References

Acuff GR, Vanderzant C, Savell JW, Jones DB, Ehlers JG (1987) Effect of acid decontamination of beef sub-primal cuts on the microbiological and sensory characteristics of steaks. Meat Sci 19 : 217-226

AOAC (1984) Official Methods of Analysis, 14 edn, Association of Official Analytical Chemists, Washington DC

Ayres JC (1960 a) Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Res 25 : 1-28

Ayres JC (1960 b) The relationships of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. J Appl Bacteriol 23 : 471-486

Banwart GJ (1981) Food spoilage In : Basic Food Microbiology, 410-450. The AVI Publishing Co, Inc. Westport. Connecticut, USA

Baumann P (1968) Isolation of *Acinetobacter* from soil and water J Bacteriol 96 : 39-42

Bouvet PJM, Grimont PAD (1986) Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov., and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii.* Int J Systematic Bacteriol 36 (2) : 228-240

Bouvet PJM, Grimont PAD (1987) Identification and biotyping of clinical isolates of *Acinetobacter.* Ann Inst Pasteur (Microbiol), 138 : 569-578

Chopade BA, Patwardhan RB, Dhakephalkar PK (1994) *Acinetobacter* infections in India : Genetic and molecular biological studies and some approaches to the problem. In : Tropical Diseases : Molecular Biology and Control Strategies, Sushil Kumar, Sen AK, Dutta GP, Sharma RN (eds) CSIR Publications and Information Directorate, New Delhi pp 704-717

Choudhari AB, Paranjape S, Sachdev R, Chopade BA (1990) Studies on occurrence of *Acinetobacter* in milk from India. FEMS workshop on the biology of *Acinetobacter* spp. abstract p 27

Cook K, Pierson MD (1983) Inhibition of bacterial spores by antimicrobials. Food Technol 37 (11) : 115-126

Dainty RH, Shaw BG, Roberts TA (1983) Microbial and chemical changes in chill-stored meats. In Food Microbiology : Advances and Prospects, Roberts TA, Skinner FA (eds). Academic Press. Inc. London. pp 151-178

Deshpande LM, Kapadnis BP, Chopade BA (1993) Metal resistance in *Acinetobacter* and its relation to β-lactamase production. BioMetals. 6(1) : 55-59

Deshpande LM, Chopade BA (1994) Plasmid mediated silver resistance in *Acinetobacter baumannii.* BioMetals. 7 : 49-56

Dhakephalkar PK, Chopade BA (1994) High levels of multiple metal resistance and its correlation to antibiotic resistance in environmental isolates of *Acinetobacter.* BioMetals. 7 : 67-74

Eribo BE, Jay JM (1985) Incidence of *Acinetobacter* spp. and other gram-negative, oxidase-negative bacteria in fresh and spoiled ground beef. Appl Environ Microbiol 49 : 256-257

Frazier WC, Westhoff DC (1995) Contamination, preservation and spoilage of meats and meat products. In : Food Microbiology, 4th edn, Tata McGraw - Hill Publishing Co. Ltd., New Delhi. pp 218-242

Gennari M, Alacqua G, Ferri F, Serio M (1989) Characterization by conventional methods and genetic transformation of Neisseriaceae

(genera *Psychrobacter* and *Acinetobacter*) isolated from fresh and spoiled sardines. Food Microbiol 6 : 199-210

Gennari M, Parini M, Volpon D, Serio M (1992) Isolation and characterization by conventional methods and genetic transformation of *Psychrobacter* and *Acinetobacter* from fresh and spoiled meat, milk and cheese. Int J Food Microbiol 15 : 61-75

Gerhardt P, Murray RGE, Wood WA, Kreig NR (1994) Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, DC USA

Gill CO, Newton KG (1980) Growth of bacteria on meat at room temperatures. J Appl Bacteriol 49 : 315-323

Gill CO, Penny N (1985) Modification in-pack conditions to extend the storage life of vacuum packaged lamb. Meat Sci 14(1) : 43-60

Gupta LK, Kalra MS, Ajit Singh, Balwant Singh (1983) Penetration of bacteria in the muscles of goat, pork and poultry meat. J Food Sci Technol 20 : 13-16

Holton J (1983) A note on the preparation and use of a selective differential medium for the isolation of *Acinetobacter* spp. from clinical sources. J Appl Bacteriol 54 : 141-142

Jay JM, Shelf LA (1978) Microbial modifications in raw and processed meats and poultry at low temperatures. Food Technol 32 : 186-187

Jay JM (1991) Modern Food Microbiology. 4th edn,. Van Nostrand Reinhold Co. New York, USA

Jensen LB (1954) Microbiology of Meats, 3rd edn, Gerrard Press, Champaign ILL, USA

Juni E (1972) Interspecies transformation of *Acinetobacter :* genetic evidence for a ubiquitous genus. J Bacteriol 112 : 917-931

Krogstad DJ, Moellering RC (1986) Antimicrobial Combinations. In Antibiotics in Laboratory Medicine, 2nd edn, Lorian V (ed), Williams & Wilkins, Baltimore, USA, pp 537-595

Lambert RJ, Strtford M (1998) Weak-acid preservatives : Modelling microbial inhibition and response. J Appl Microbiol 86 : 157-164

Narasimha Rao D, Sreenivasamurthy V (1985) A note on microbial spoilage of sheep meat at ambient temperature. J Appl Bacteriol 58 : 457-460

Narasimha Rao D, Nair KKS, Sakhare PZ (1998) Meat Microbiology and spoilage in tropical countries. In : The Microbiology of Meat and Poultry. Andrew Davies, Ron Board (eds.). Blackie Academic and Professional, London, pp 221-165

Ogbadu LJ (1999) Permitted preservatives - Benzoic acid. In Encyclopedia of Food Microbiology Vol. 3 : Robinson RK, Batt CA, Patel P (eds) Academic Press Inc, Manhattan, New York, USA pp 1754-1757

Russel AD, Hugil WB, Ayliffe GAJ (1982) Common uses of chemical food preservatives In Principal and practice of disinfection and sterilisation. Blackwell Scientific Publications, Oxford, London, Edinburgh, p 312

Saha SC, Chopade BA (2001) Studies on occurrence and distribution of *Acinetobacter* spp. and other gram-negative bacteria from meat. J Food Sci Technol 38 (1) : 17-22

Sankaran R, Murali HS, Leele RK, Sharma TE (1986) Enrichment of colour and texture of mutton by the use of lactic acid bacteria. J Food Sci Technol 23 : 172-175

Shakibaie MR, Kapadnis BP, Dhakephalkar PK, Chopade BA (1999) Removal of silver from photographic waste water effluent using *Acinetobacter baumannii* BL54. Can J Microbiol 45 : 959-1000

Singhal RS, Kulkarni PR (1999) Permitted preservatives - Nitrite and nitrate in Encyclopedia of Food Microbiology Vol. 3 : Robinson RK, Batt CA, Patel P (eds) Academic Press Inc, Manhattan pp 1762-1769

Stratford M, Anslow PA (1998) Evidence that sorbic acid does not inhibit yeast as a classic weak acid preservative. Letters Appl Microbiol 27 : 203-206

Surekha M, Reddy S (1999) Preservatives/Classification and properties in Encyclopedia of Food Microbiology Vol. 3 : Robinson RK, Batt CA, Patel P (eds) Academic Press Inc, Manhattan, New York, USA p 1710

Thomas LV (1999) Permitted preservatives - Sorbic acid. Aplin and Barrett Ltd (Cultor Food Science), Beaminster, UK. In : Encyclopedia of Food Microbiology Vol. 3 : Robinson RK, Batt CA, Patel P (eds) Academic Press Inc, Manhattan, New York, USA pp 1769-1776

Vijaya Rao D, Bhagirathi B, Sharma TR (1983) Fluctuations in the quantitative predominance of bacterial groups in fresh stored mutton. J Food Sci Technol 20 : 277-281

Walkenbach J (1995) Excel 5 for Windows Power Programming Techniques, 2nd ed, IDG Books Worldwide, Inc., San Mateo, California, USA

Ziauddin KS, Rao DN, Amla BL (1993) In vitro study on the effect of lactic acid and sodium chloride on spoilage and pathogenic bacteria of meat. J Food Sci Technol 30 : 3, 204-207

Received 19 August 2000; revised 4 July 2001; accepted 9 December 2001

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 33-37

Effect of Primary Processing on Microbial Load of Cauliflower and Fenugreek

SAJI GOMEZ*[1], LATA[2] AND SUSANTA K, ROY[3]

[1,3] Division of Fruits and Horticultural Technology,

[2] Division of Microbiology,

Indian Agricultural Research Institute, New Delhi - 110 012, India.

The influences of packaging material (shrink wrap), storage atmosphere (ambient, cool chamber and cool store) and antimicrobial (potassium metabisulphite) on the quality of primary processed cauliflower and fenugreek were studied. The results showed that washing cauliflower florets in tapwater reduced the microbial load as compared to the unwashed samples. The tapwater washed florets when further treated with potassium metabisulphite had lower microbial counts as compared to the florets washed in tapwater. Baskets containing florets when shrink-wrapped and stored at low temperature (cool chamber and cool store) resulted in lower microbial proliferation. The wrapped florets treated with potassium metabisulphite had lower microbial counts than the unwrapped florets without potassium metabisulphite. Similarly, fenugreek leaves when shrink-wrapped and stored at low temperatures resulted in lower microbial growth as compared to unwrapped samples stored at ambient temperature.

Keywords : Bacteria, Cauliflower, Cool chamber, Cool store, *Escherichia coli,* Fenugreek, Fungi, Microbial load, Potassium metabisulphite, Precooling, Primary processing, Shrink wrapping.

Convenience, freshness and ready-to-use nature of primary processed vegetables have led to their increasing demand in recent years. Primary processing involves removal of inedible parts, washing, sorting, trimming, slicing or chopping that do not affect the fresh-like quality of the vegetable. Such procedures provide convenient ready-to-use fresh products that can be prepared and consumed in less time. But these lightly processed vegetables can be contaminated with many spoilage organisms including yeasts, moulds and bacteria because of their moisture content. Furthermore, some of the bacteria that may be present, including *Clostridium botulinum* and *Listeria monocytogenes* are pathogenic to consumers (O'Beirne 1990). Packaging primary processed produce in permeable polymeric film, semi-rigid containers or both can reduce oxygen concentration and increase carbon dioxide concentration in the package atmosphere, thereby slowing degradative quality changes and increasing product shelf life. The present investigation was undertaken to study the effect of primary processing on microbial load of cauliflower and fenugreek.

Materials and Methods

Cauliflower variety 'Pusa Snowball' and fenugreek variety 'Push Early Bunching' were harvested at the optimum edible stage from the vegetable demonstration plot of IARI and were quickly transported to the laboratory in plastic crates.

Primary processing : Cauliflower curds, after the removal of inedible parts were cut into florets and these florets were washed in tap water. The tap water washed florets were divided into two lots. One lot was dipped in potassium metabisulphite (KMS) solution (3000 ppm) for 30 min and the other lot was left as such. The treated and untreated (tap water washed) florets were spread on perforated aluminium trays to drain out excess water. These florets were again kept in a cabinet drier maintained at 40^{0}C for 20 min to remove the surface moisture. After the removal of surface moisture, about 250 g florets were filled in each perforated plastic basket, half of the baskets containing sulphited florets and the other half containing tap water washed florets. These baskets containing sulphited and tapwater washed florets were precooled in a cool store maintained at 2-5^{0}C for 30 min. After precooling, half of the baskets containing sulphited florets were overwrapped with cling film and the other half were left unwrapped. Likewise, half of the baskets containing tapwater washed florets were overwrapped with cling film and the other half were left unwrapped. Baskets (wrapped and unwrapped) containing florets (treated and untreated) and the whole curds (control samples) were stored at room temperature (RT), cool chamber (CC) (Roy 1997) and cool store (CS).

In case of fenugreek, the leaves were separated from the bunches and unlike cauliflower florets, they were not subjected to washing as washing of leaves resulted in loss of turgidity. The leaves were then spread on perforated trays to remove traces of moisture from the leaf surface. About 100 g leaves were filled in each perforated plastic basket and was precooled in a cool store maintained at 2-5^{0}C for 30 min. After precooling, half of the baskets containing leaves were overwrapped with cling film and the other half were left unwrapped. The wrapped and unwrapped baskets containing leaves, along with the whole bunches (control samples) were stored at room temperature, cool chamber and cool store. The temperature and relative humidity under the three storage conditions are given in Table 1.

Microbiological analysis : The samples were subjected to microbiological analysis initially and as and when the symptoms of spoilage were spotted. The samples were analysed for the population of bacteria, fungi and presence of *E. coli* in standard plate count agar (Harrigan and Mc Cane 1966). Martin rose bengal agar (Martin 1950) and BCP lactose broth media (Seeley and Van Denmark 1970), respectively.

*[1] *Corresponding Author*

Appropriate dilutions were plated on respective media and incubated at 30°C for 24-48 h. The results are expressed in terms of cfu/g of sample. For *E. coli*, 10^{-1} and 10^{-2} dilutions were inoculated in BCP-lactose broth tubes and incubated at 37°C for 48 h and observations were taken for acid and gas production. If tubes recorded positive for acid and gas, then it was considered positive for the presence of coliforms.

Results and Discussion

The results showed that cauliflower and fenugreek supported the growth of both bacteria and fungi. High moisture and nutrient contents in addition to a neutral pH make vegetables capable of supporting the growth of almost any type of microorganism. In general, vegetables are about equally contaminated with bacteria and fungi (Brackett and Splittstoesser 1992). In the present investigation, it was observed that washing cauliflower florets in tap water brought about 25 % reduction in bacterial population. Further dipping the washed florets in potassium meta bisulphite (KMS) solution again reduced the population marginally (Table 2). Similarly, washing the florets in tap water reduced fungal population up to 50% Sulphitation of the washed florets further reduced the load of fungi (Table 3). This finding was in conformity with that of Ru-Yin Chen et al (1997) who reported that washing reduced the total microbial count of cut chive by 0.50-0.70 log cfu/g. Sulphurdioxide has been used to control microorganisms in the fruit and vegetable realm on soft fruits and leafy greens and is also well known to be effective against moulds, yeasts and bacteria (Dziezak 1986).

In the present investigation, it was found that the bacterial population increased in all the treatments during storage of cauliflower (Tables 2 and 3). During the first five days, the growth was slow and then, it increased and the maximum growth was observed on the ninth day. Among the whole curds (control samples), the samples stored at ambient temperature had the highest bacterial counts followed by the curds stored in cool chamber and cool store. The curds (control samples) stored at ambient temperature and cool chamber had to be

TABLE 1. STORAGE TEMPERATURE (°C) AND RELATIVE HUMIDITY (%) CONDITIONS IN THE STORAGE ENVIRONMENT DURING STORAGE STUDIES

Month	Ambient		Cool chamber		Cool store	
	Temperature range,°C	RH range, %	Temperature range,°C	RH range, %	Temperature range,°C	RH range, %
November	12.0-27.0	60-80	13.0-24.0	86-92	2-5	65-70
December	6.9-22.0	60-80	10.0-20.0	86-92	2-5	65-70
January	7.8-17.7	60-80	10.0-15.0	86-92	2-5	65-70
February	9.5-22.3	60-75	13.0-17.0	90-95	2-5	65-70
March	12.0-30.3	40-80	17.0-20.5	90-93	2-5	65-70

TABLE 2. CHANGES IN BACTERIAL POPULATION OF CAULIFLOWER DURING DIFFERENT STAGES OF STORAGE

Treatments		Bacterial population x 10^5 cfu/g				
		Initial	5 days	9 days	16 days	23 days
Whole curd (RT)	T_1	23.067	29.100	35.767*	-	-
Whole curd (CC)	T_2	23.067	25.600	36.300*	-	-
Whole curd (CS)	T_3	23.067	23.500	27.100	26.000	18.933
Florets+RT+unwrapped	T_4	17.633	20.600*	-	-	-
Florets+CC+unwrapped	T_5	17.633	18.233*	-	-	-
Florets+CS+unwrapped	T_6	17.633	15.867	22.133	21.467	13.967
Florets+KMS+RT+unwrapped	T_7	13.700	17.700*	-	-	-
Florets+KMS+CC+unwrapped	T_8	13.700	15.133	22.800*	-	-
Florets+KMS+CS+unwrapped	T_9	13.700	14.433	18.833	18.233	10.567
Florets+RT+wrapped	T_{10}	17.633	19.133	23.500*	-	-
Florets+CC+wrapped	T_{11}	17.633	18.600	21.600*	-	-
Florets+CS+wrapped	T_{12}	17.633	17.967	19.367	17.867	10.300
Florets+KMS+RT+wrapped	T_{13}	13.700	15.600	19.800	19.000*	-
Florets+KMS+CC+wrapped	T_{14}	13.700	15.500	18.367	17.600	13.867
Florets+KMS+CS+wrapped	T_{15}	13.700	14.500	15.400	14.567	8.633
SEm ±		0.581	0.954	0.609	0.332	0.299
CD at 5%		1.670	2.741	1.748	0.955	0.859

*Removed due to mould development. RT = Room temperature. CC = Cool chamber. CS = Cool store

TABLE 3. CHANGES IN FUNGAL POPULATION OF CAULIFLOWER DURING DIFFERENT STAGES OF STORAGE

Treatments		Fungal population, x 10^3 cfu/g				
		Initial	5 days	9 days	16 days	23 days
Whole curd (RT)	T_1	5.633	8.900	13.767	-	-
Whole curd (CC)	T_2	5.633	8.533	17.067	-	-
Whole curd (CS)	T_3	5.633	6.400	7.000	12.533	17.667
Florets+RT+unwrapped	T_4	3.833	5.867*	-	-	-
Florets+CC+unwrapped	T_5	3.833	5.600*	-	-	-
Florets+CS+unwrapped	T_6	3.833	4.800	5.833	9.867	17.967
Florets+KMS+RT+unwrapped	T_7	3.033	5.033*	-	-	-
Florets+KMS+CC+unwrapped	T_8	3.033	4.900	6.467	-	-
Florets+KMS+CS+unwrapped	T_9	3.033	3.567	4.433	6.867	10.833
Florets+RT+wrapped	T_{10}	3.833	5.700	7.633*	-	-
Florets+CC+wrapped	T_{11}	3.833	4.833	6.933*	-	-
Florets+CS+wrapped	T_{12}	3.833	4.267	5.167	5.467	11.233
Florets+KMS+RT+wrapped	T_{13}	3.033	4.400	6.000	10.100*	-
Florets+KMS+CC+wrapped	T_{14}	3.033	4.233	5.433	9.667	14.833
Florets+KMS+CS+wrapped	T_{15}	3.033	3.533	4.333	5.900	9.567
SEm ±		0.226	0.244	0.213	0.311	0.834
CD at 5%		0.649	0.701	0.611	0.911	1.117

*Removed due to mould development. RT = Room temperature. CC = Cool chamber. CS = Cool store

TABLE 4. CHANGES IN BACTERIAL POPULATION OF FENUGREEK DURING DIFFERENT STAGES OF STORAGE

Treatments		Bacterial population, x 10^5 cfu/g			
		Initial	4 days	9 days	13 days
Whole bunch (RT)	T_1	4.37	15.43*	-	-
Whole bunch (CC)	T_2	4.37	12.46	19.26	18.23
Whole bunch (CS)	T_3	4.37	7.33	8.63*	-
Leaves+RT+unwrapped	T_4	4.37	10.53*	-	-
Leaves+CC+unwrapped	T_5	4.37	8.33	15.00*	-
Leaves+CS+unwrapped	T_6	4.37	5.93	6.00*	-
Leaves+RT+wrapped	T_7	4.37	5.90	11.50*	-
Leaves+CC+wrapped	T_8	4.37	5.63	10.53	9.87
Leaves+CS+wrapped	T_9	4.37	5.20	7.63	7.47
SEm ±		0.43	0.60	0.48	0.31
CD at 5%		1.22	1.73	1.40	0.90

*Unmarketable. RT = Room temperature. CC = Cool chamber. CS = Cool store

removed after 9 days due to fungal spoilage. Among the unwrapped florets, the sulphited samples kept in cool store had the lowest bacterial load. The rate of bacterial proliferation was slow in the sulphited wrapped florets in cool store. All the samples stored in cool chamber had comparatively less bacterial proliferation than the room temperature stored samples.

Fungal proliferation also showed a similar trend in the samples stored at the three different storage conditions (Table 3). Maximum fungal population was recorded on the 9th day on the whole curd (control sample) stored at room temperature. The fungal population was minimum on florets, which were treated with KMS and kept in cool store under wrapped conditions. At this stage, some of the samples showing visible spoilage due to fungal growth were removed. The experiment was continued upto 23 days. Variation was observed in fungal population among the treatments. Sulphited wrapped samples under the three storage conditions experienced retarded fungal proliferation as compared to their non-sulphited and unwrapped counterparts. It can be concluded from the results (Tables 2 and 3) that sulphited florets can be stored in cool store upto 23 days with minimum proliferation of bacteria and fungi.

In case of fenugreek (Tables 4 and 5), bacterial population was less as compared to cauliflower and its number increased upto the 9th day. The bacterial and fungal load was maximum in the whole bunch (control sample) stored at room temperature and lowest in wrapped leaves in cool store. Wrapping provided substantial improvement in extending shelf life. The leaves could be stored upto 13 days in wrapped conditions unlike

TABLE 5. CHANGES IN FUNGAL POPULATION OF FENUGREEK DURING DIFFERENT STAGES OF STORAGE

Treatments		Fungal population, x 10^5 cfu/g Initial	4 days	9 days	13 days
Whole bunch (RT)	T_1	2.33	4.33*	-	-
Whole bunch (CC)	T_2	2.33	3.68	6.50	3.86
Whole bunch (CS)	T_3	2.33	2.40	2.56	-
Leaves+RT+unwrapped	T_4	2.33	3.90*	-	-
Leaves+CC+unwrapped	T_5	2.33	3.32	6.13	-
Leaves+CS+unwrapped	T_6	2.33	1.80	1.67	-
Leaves+RT+wrapped	T_7	2.33	3.37	7.07	-
Leaves+CC+wrapped	T_8	2.33	2.97	6.33	5.20
Leaves+CS+wrapped	T_9	2.33	2.23	4.67	3.13
SEm ±		0.28	0.16	0.19	0.11
CD at 5%		0.82	0.45	0.54	0.30

*Unmarketable. RT = Room temperature. CC = Cool chamber. CS = Cool store

9 days in unwrapped conditions. It can be concluded that wrapping of samples hinders microbial proliferation to a considerable extent at low temperature. Wrapped and unwrapped samples when stored at room temperature resulted in higher microbial proliferation. This might be because the presence of droplets as well as high temperature prevailing in room favoured the growth of microbes. Storage of fruits and vegetables in high humidity allows droplets of condensed moisture to accumulate on the product and this accumulated liquid can allow any microorganism present to spread more easily to other items and may also serve as a growth broth (Brackett 1987; Lund 1971).

When the results of Tables 3 and 5 are compared, it is evident that fungal population is more in fenugreek as compared to cauliflower. This may be because of the fact fenugreek was not washed. This finding is in accordance with that of Van den Berg and Lentz (1996) who have reported that mildly dehydrated produce is more successfully infected by some microbes. Fungal population was maximum in room temperature stored samples followed by the samples in cool chamber and cool store. However, wrapped samples supported the lowest fungal population. Low humidity during storage can also favour development of fungi, which tolerate low water activity environments (Brackett 1987). All the samples were tested for the presence of coliforms and none of the cauliflower / fenugreek samples tested positive for the presence of *E. coli.*

Wrapping of cauliflower florets and fenugreek leaves and their subsequent storage in cool store was found to be the best method to minimize microbial proliferation in primary processed cauliflower and fenugreek. This finding is in agreement with that of Bennik et al. (1996) who reported that the use of modified atmosphere packaging and refrigeration temperature could reduce the microbial spoilage of cut endive and improved conditions for the safety of the product. Earlier reports on the beneficial effects of modified atmospheric storage on reduced development of mould in broccoli florets (Sylvie Bastrash et al. 1993) and aerobic microflora in lightly processed fresh endive (Carlin et al. 1996) confirm the present findings. Modified atmosphere packaging of shredded carrot and cabbage in polypropylene film and when stored at 7^{0}C, decreased the number of all pathogens on day zero and also on the eighth day (Fiun and Upton 1997).

Conclusion

From the present study, it can be concluded that the presence of moisture and humidity plays very important role in the survival and proliferation of bacteria and fungi. But washing, sulphitation and wrapping along with storage at low temperature can be considered as an efficient treatment for improving the shelf life of primary processed vegetables.

References

Bennik MHJ, Pepplenbos HW, Nguyen-the C (1996) Microbiology of minimally processed, modified atmosphere packaged chicory endive. Post-harvest Biol Technol 9(2): 209-221

Brackett RE (1987) Microbiology consequences of minimally processed fruits and vegetables. J Food Qual 19: 195-206

Brackett RE, Splittstoesser DF (1992) Fruits and vegetables. In: Compendium of Methods in Microbiological Examination of Foods. 3rd edn., Splittstoesser F, Vanderzant C (eds). American Public Health Association, Washington, DC pp 919-927

Carlin F, Nguyen-the C, Abreu da Silva A, Cochet C (1996) Effects of carbondioxide on the fate of *Listeria monocytogenes* of aerobic bacteria and on the development of spoilage in minimally processed fresh endive. Intt J Food Microbiol 32(1/2): 159-172

Dziezak JD (1986) Preservative systems in foods, antioxidants and antimicrobial agents. Food Technol 40(9): 94-136

Fiun MJ, Upton ME (1997) Survival of pathogens on modified atmosphere packaged shredded carrot and cabbage. J Food Protection 60(11): 1347-1350

Harrigan WF, Mc Cance ME (1966) Laboratory Methods in Microbiology Academic Press, New York, p. 362

Lund BM (1971) Bacterial spoilage of vegetables and certain fruits J Appl Bacteriol 34 : 9-20

Martin JR (1950) Use of acid, rose bengal and streptomycin in the

plate method for estimating soil fungi. Soil Sci 69: 215-217

O'Beirne D (1990) Modified atmosphere packaging of fruits and vegetables. In : Chilled Foods. The State-of-Art. Gormley (ed) Elsevier Science Publishing Co., New York, pp. 183-199

Roy Susanta K (1997) Low-cost, environment friendly cool chamber-A Technology for 2020. ICAR News 3(4): Oct-Dec issue

Ru-Yin Chen Ming-Sai Liu, Meei-Ju Tasi (1997) Effects of washing and storage temperature on microbial counts of freshly cut chive and lettuce. Food Sci Taiwan 24(3): 357-364

Seeley HW Jr, Van Demark PJ (1970) Microbes in action:A Laboratory Manual of Microbiology. WH Freeman and Co. New York p 178

Sylvie Bastrash, Goseph Makhlouf. Francois Castaigve, Claude Willemot (1993) Optimal controlled atmosphere conditions for storage of broccoli florets. J Food Sci 58(2): 338-341

Van den Berg L, Lentz CP (1996) Effect of temperature, relative humidity and atmosphere composition on changes in quality of carrots during storage. Food Technol 20: 104-107

Received 28 November 2000; revised 9 July 2001; accepted 9 December 2001

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 38-41

Mycotoxins, Mycotoxigenic fungi and the Biochemical Changes in *Makhana* (*Euryale ferox* Salisb) Puffs of North Bihar

BASANT K SINHA*, K.S. RANJAN AND T.N. PANDEY

Department of Veterinary Microbiology,
Bihar Veterinary College, Patna - 800 014, India.

A survey was conducted in large areas of North Bihar and Ganga basin for mycotoxigenic infestation in *makhana* puffs. About 12 types of fungal species have been isolated in which *Aspergillus flavus* groups, *A. ochraceus* and *Penicillium citrinum* have been tested for their mycotoxin producing capabilities. About 30% of *makhana* puffs samples were contaminated with aflatoxins, ochratoxins and citrinin. Upto 12 µg/g of aflatoxin B_1 has been elaborated in makhana puffs and significant changes were observed in their nutritional components.

Keywords : Aflatoxin, Ochratoxin, Citrinin, Makhana puffs.

Fungi elaborate a wide range of toxic secondary metabolites on food known as mycotoxins, which are of great concern due to their adverse effects on animals, plants and different microbial systems. The problem of mycotoxigenic infestation on *makhana* (*Euryale ferox* Salisb) is due to its primitive way of post-harvest and storage practices including conducive climatic conditions of Bihar regions. Therefore, a systematic survey for mycotoxin contamination in makhana puffs have been carried out, as makhana cultivation comes under main agricultural economy.

Aflatoxin elaboration by *Aspergillus flavus* depends upon the nature of substrates. Therefore, the level of aflatoxin produced by toxigenic strains of *A. flavus* on makhana puffs was also studied. Besides, producing aflatoxins, these strains also cause considerable damage to the nutritive value of the substrates by changing their chemical constituents (Singh and Sinha 1982). An attempt has been made to evaluate the quantitative changes in their nutritional contents during aflatoxin elaboration by *A. flavus.*

Materials and Methods

Altogether 39 samples of *makhana* puffs were collected from different villages and markets of North Bihar. These samples were processed for fungal isolation by Blotter test and agar plate method (ISTA 1966). Percent incidence of fungi was determined on the basis of occurrence of a particular fungal species in hundred samples. Aflatoxin producing potentials of *Aspergillus flavus* group fungi was tested in SMKY liquid medium (Diener and Davis 1966). The methods of Davis et al (1972) and Schwenk et al (1958) were followed for ochratoxin A and citrinin producing abilities of *Aspergillus ochraceus* and *Penicillium citrinum* isolates, respectively.

The collected samples (50g each) were dried at 60°C for nearly 48 h and separately ground for chemical extractions of different mycotoxins (Roberts and Patterson 1975). For the extraction of citrinin, the aqueous acetonitrile phase was acidified with 1M HCL and finally extracted with chloroform, which was evaporated to near dryness on a water bath and the residue was transferred to screw capped vials for TLC.

**Corresponding Author*

The detection of mycotoxins was carried out by thin layer chromatography (TLC). Two solvent systems i.e. T.E.F (toluene/ethyl acetate/formic acid, 6:3:1, v/v) and BMA (benzene/methanol/acetic acid, 24:2:1, v/v) were used for the development of the chromatoplate. For aflatoxins, another solvent system, toluene/isoamyl alcohol/methanol (90:32:2, v/v), was used (Reddy et al. 1970).

Chemical confirmation of aflatoxins was performed with trifluoroacetic acid (Stack and Pohland 1975) and by spraying the chromatoplate with 25% sulphuric acid. The presence of ochratoxin A on the TLC plate was confirmed by treatment with ammonia (Davis et al. 1969) whereas citrinin was confirmed by spraying with an acidic solution of p-anisaldehyde (Scott et al. 1970).

The quantity of aflatoxin B_1 present in the sample was determined spectrophotometrically (Nabney and Nesbitt 1965) and wherever needed, fluorodensitometer was used. Amounts of citrinin and ochratoxin were calculated visually on TLC plate by comparing known concentration of standard solution (50 to 1000 µl/L).

Aflatoxin elaboration on makhana puffs : Healthy samples (50 g) were soaked in distilled water and subsequently autoclaved for 10 min at 15 lbs psi. On the following day, these were inoculated separately with 0.5 ml spore suspension of known toxigenic strain of *A.flavus*. Each set was run in triplicate and all the flasks were inoculated at 28 ± 1°C for 11 days. On the 12th day, the spores of infested material were killed by adding 5 ml methanol and then dried at 60°C for 48 h and crushed subsequently. Aflatoxins were extracted and estimated by the methods described earlier.

Chemical change in makhana puffs during aflatoxin elaboration : For this, *makhana* puffs were soaked in 2% sodium hypochlorite solution for 10 min, subsequently washed with sterilized distilled water for 3 to 4 times. The methods of inoculation and incubation were same as followed for aflatoxin elaboration. However, in this case, puffs were inoculated separately with two known toxigenic and non-toxigenic strains of *A. flavus*. After incubation, the infested puffs were dried at 60°C for 48 h and were powdered subsequently.

Changes in the quantity of total sugars were estimated by the methods of Dubois et al. (1956) and reducing sugars by Somogy-Nelson method (Plummer 1971). Amount of non-reducing sugars was however, determined by substracting the value of reducing sugars from total sugars. Protein contents of the infected puffs were determined colorimetrically by the method of Lowry et al. (1951), whereas ascorbic acid content of puff tissue was determined by the method of Roe and Kuether (1943). Phenol contents were estimated, following the method of Singh et al. (1978).

Data were statistically analysed. Mean values along with standard deviation were calculated for this purpose. "t" test value was also calculated and their percent significance was recorded using ANOVA.

Results and Discussion

Altogether 12 types of fungi were recorded from the *makhana* puff samples. These included : *Aspergillus flavus* link ex Fries (100%), *A.niger* van Tiegh (37%), *A.ochraceus* Wilhelm (32%), *A.fumigatus* Fres. (11%), other *Aspergillus* sp. (21%). *Penicillium citrinum* Thom (42%), *Penicillium oxalicum* Thom (16%), *Fusarium* sp. (11%), *Rhizopus stolonifer* Ehrenb Ex Fr. (11%), *Actinomucor repens* Schost (6%), *Curvularia lunata* (Wak) Boed (5%) and *Chaetomium globosum* Kunze (3%).

Table 1 shows the results of screening of fungal isolates for their toxin producing ability. Out of 164 isolates of *A. flavus* group, 85 were aflatoxin producers which produced different components of aflatoxins. Aflatoxin B_1 alone was elaborated by the maximum number of isolates (34), whereas 26 isolates produced AFB_1 and B_2. 21 isolates yielded AFG_1 alongwith B_1 and B_2, while all the four aflatoxin viz, B_1, B_2 G_1 and G_2 were synthesized by 4 isolates only. The percent toxigenicity among other mycotoxin producing fungi was comparatively lower. None of the 9 isolates of *A. fumigatus* produced any mycotoxin, whereas 17 isolates (out of 45) of *A. ochraceus* produced ochratoxin A. Citrinin was produced by 6 of 27 *Penicillium citrinum* isolates screened.

TABLE 1. MYCOTOXIN PRODUCING POTENTIALS OF THE ISOLATED FUNGI

Fungi	No. of isolates screened	Toxigenic isolates	Toxigenicity, %	Mycotoxin produced	Range of concentration, µg/ml
Aspergillus	164	85	51	Aflatoxins	0.2 - 16
flavus group		34		AFB_1	
		26		AFB_1, B_2	
		21		AFB_1, B_2, G_1	
		04		AFB_1, B_2, G_1, G_2	
A.fumigatus	09	-	-	-	-
A.ochraceus	45	17	37	Ochratoxin A	0.1 - 0.4
Penicillium citrinum	27	06	22	Citrinin	0.15 - 0.6

TABLE 2. NATURAL INCIDENCE OF DIFFERENT MYCOTOXINS IN MAKHANA PUFFS

No. of samples analysed	No. of samples contaminated with mycotoxin	Specific Mycotoxin detected Mycotoxins	No. of positive samples	Concentration of mycotoxins, µg/kg
39	12 (30.76)	Aflatoxin	7 (17.94)	100 - 2140
		Ochratoxin	3 (7.69)	100 - 600
		Citrinin	2 (5.12)	100 - 500
		Aflatoxin + Ochratoxin	2 (5.12)	

Values in the parenthesis are the percent contamination of the total number of samples screened.

The amount of aflatoxins produced by the toxigenic isolates was in the range of 0.2-16 µg/ml (Table 1). On the basis of the capacity to produce aflatoxin B_1, these were grouped as high and low toxin producers. Thirty five isolates of *A. flavus* were high producers, which produced AFB_1 in the range of 5-15 µg/ml, whereas 59 isolates were low producer and these were able to elaborate AFB_1 only upto 5 µg/ml. The range of ochratoxin A and citrinin were 100-400 and 150-600 µg/litre respectively.

Thirty nine samples of *makhana* were subjected to various physico-chemical test for natural contamination of mycotoxin (Table 2). Aflatoxins, the most common mycotoxin encountered as natural contaminants in makhana puff samples, were detected in twelve samples. Frequency and occurrence of ochratoxin A and citrinin were comparatively lesser. In 2 contaminated samples, aflatoxin was found alongwith ochratoxin A. Amount of aflatoxin B_1 in the contaminated samples ranged between 100-2140 µg/kg. The levels of ochratoxin A and citrinin were 100-600 and 100-500 µg/kg respectively.

Aflatoxin elaboration by *A.flavus* on makhana puffs was also tested. *Makhana* puff was found to be a good substrate for aflatoxin elaboration. Upto 1200 µg/kg of AF B_1 was found to be elaborated.

The changes in various chemical components in makhana puff, due to infestation with toxigenic and non-toxigenic strains of *A.flavus* are depicted in Table 3. Total sugars were reduced by 48.08 and 41.35%, respectively by the toxigenic and non-toxigenic strains. Fall in the levels of reducing sugars, non-reducing sugars and ascorbic acid was 79.66, 31.78 and 71.94%, respectively, by the toxigenic strain. However, increases in protein and phenol contents by 68.78 and 97.62%, respectively, were recorded due to this strain. Non-toxigenic strain of *A.flavus* caused maximum depletion in ascorbic acid which was upto 78.87%. Protein and phenol levels, however, got increased by 425.55 and 219.05%, respectively.

The results indicated that mycotoxins and mycotoxigenic fungi were in *makhana* puffs. A high incidence of *Aspergillus flavus* had earlier been reported in the foodgrains and feeds of this region (Sinha and Ranjan 1990). The presence of other fungal species in association with *A.flavus* was of ecological significance. Isolation of these fungi is not always related to

TABLE 3. CHANGES IN THE LEVEL OF SUGARS, PROTEIN, ASCORBIC ACID AND PHENOL IN MAKHANA PUFF DURING AFLATOXIN ELABORATION

Constituent	Amount %				"t" test	
	Control Puff		*Aspergillus flavus* infested puff			
	0 day	11 days	Toxigenic	Non-toxigenic	AFT	AFNT
Total sugars	11.20	10.40	5.40	6.10	170.65*	183.76*
	± 0.02	± 0.036	± 0.036	± 0.02		
Reducing	3.70	3.54	0.72	0.96	115.10*	105.30*
sugars	± 0.056	± 0.03	± 0.03	± 0.03		
Non-reducing	7.50	6.86	4.680	6.140	134.57*	106.17*
sugars	± 0.04	± 0.01	± 0.026	± 0.026		
Proteins	0.97	0.900	7.09	4.730	196.50*	188.67*
	± 0.02	± 0.026	± 0.05	± 0.033		
Ascorbic	1.05	0.980	0.275	0.212	275.39*	480.00*
acid	± 0.03	± 0.002	± 0.004	± 0.002		
Phenol	0.195	0.210	0.415	0.670	30.59*	69.13*
	± 0.004	± 0.004	± 0.003	± 0.008		

AFT = *A.flavus* toxigenic; AFNT = *A.flavus* non-toxigenic; *=$p < 0.01$

mycotoxin contamination (Moreau 1979).

The results further revealed that although mycotoxin producing capacity was exhibited by several fungi. *A. flavus* group had varied capacity to elaborate different components of aflatoxins i.e., AFB_1, B_2, G_1 and G_2. Aflatoxin B_1 was invariably produced by all the toxigenic isolates of *A. flavus.* However, the frequency of *A. flavus* isolates to produce aflatoxins other than AFB_1 was comparatively low (Ranjan 1986). All the strains of *A. ochraceus* were not capable of producing ochratoxin A. Similar was the case with regard to citrinin production by *Penicillium citrinum.* In this investigation, only 37% isolates of *A. ochraceus* produced ochratoxin A whereas 22% isolates of *P. citrinum* elaborated citrinin.

Natural association of mycotoxin producing fungi with *makhana* puff samples would elaborate mycotoxins in congenial condition. Out of 39 samples analysed, 30% were found to be contaminated with different mycotoxins. Aflatoxins were the most frequent mycotoxins to be encountered. Frequency of occurrence of ochratoxin A and citrinin were comparatively lesser. The co-occurrence of aflatoxin with other mycotoxin is due to the colonisation by more than one species of mycotoxigenic fungi, which could produce different mycotoxins. Ranjan and Sinha (1991), also detected aflatoxin, zearalenone and ochratoxin A together in animal feed samples.

The high incidence of mycotoxins, in particular aflatoxins, in collected samples might be attributed to several environmental and socio-agronomic factors. Temperature and humidity play important roles in producing natural contamination in mycotoxins. Climatic conditions in large areas of North Bihar and Ganga basin are highly conducive to mycotoxin production.

The farmers caused mechanical injury during picking of the puffs from its hard cover shell, and subsequently leave the harvested crops in the open field for sun drying. Mycotoxigenic fungi are well distributed in the surrounding area and easily invade the puffs at this stage. During storage, puffs are kept in gunny bags where chance of storage insects as mechanical vectors for mycotoxigenic conidia are easier. The accumulation of large number of conidia of *A. flavus* and other mycotoxigenic fungi is the major factor for higher accumulation of toxins. It is important to note that all the contaminated samples contained AFB_1 by more than 20 µg/ml, and the tolerance level fixed by WHO is less than 20 µg/ml. Such a high concentration of mycotoxins and specially the aflatoxins may have deleterious effects on the consumer population (Ranjan 1985).

Apart from natural contamination of various mycotoxins, the level of aflatoxin elaboration was evaluated under laboratory conditions also. *A.flavus* elaborated aflatoxin B_1 in makhana puffs upto 1200 µg/kg. High level of aflatoxin produced by the toxigenic strain of *A.flavus* in makhana is obviously due to their rich nutritional components (carbohydrates 76.9%, proteins 9.7%, fat 0.1% and minerals 0.5%). High carbohydrates content appears to be responsible for high level production of aflatoxin (Misra et al. 1980).

Moulds are also known to degrade the nutritive quality of the food materials during mycotoxin production. The fungi modify the substrates on which they grow and use some of their chemical components required for the growth and metabolic processes. Many of them also produce powerful enzymes capable of degrading the food constituents. The chemical changes in makhana puffs due to infestation with toxigenic and non-toxigenic strains of *A. flavus* were also recorded. A sharp decline in the levels of total, reducing and non-reducing sugars was noted. Toxigenic strain of *A. flavus* utilized greater quantity of sugars during its metabolism as compared to non-toxigenic

strains. This is possible due to the presence of more glycolytic enzymes in the toxigenic strain which hydrolyse sugar molecules to simpler form like pyruvate, which is central precursor for aflatoxin biosynthesis (Bilgrami et al. 1983) Increase in protein content during infestation by *A. flavus* was quite distinct. Earlier report also indicated that protein accumulation was due to fungal infestation as well as subsequently to aflatoxin production (Cherry et al. 1974). Although there is no evidence of the direct role of ascorbic acid or phenol in aflatoxin biosynthesis, changes in their amount may be due to fungal infestation. The degree of increase or decrease, however, depends upon the nature of *A. flavus* strain (Sinha and Singh 1982). As makhana is the main agricultural cash crop of North Bihar specially in the Koshi region and north of the Ganga basin, every possible effort is to be made to minimize the level of mycotoxins.

Conclusion

Makhana puffs have been found to be a good substrate for the growth of different mycotoxigenic fungi. Present investigation revealed the presence of aflatoxins, ochratoxin and citrinin on makhana samples which may cause in serious health problem. As *makhana* is one of the exporting materials of Bihar, its quality and nutritional status must be maintained by applying recommended control programmes of our country.

References

Bilgrami KS, Sinha KK, Singh A (1983) Chemical changes in dry fruits during aflatoxin elaboration by *Aspergillus flavus* Link Ex Fries. Curr Sci 52 : 960-964

Cherry JP, Mayne RY, Ory RL (1974) Proteins and enzymes from seeds of *Arachis hypogea* L IX. Electrophoretically detected changes in 15 peanut cultivars grown in different areas after inoculation with *Aspergillus parasiticus*. Physiol Plant Pathol 4 : 425-435

Davis ND, Sansing GA, Ellenburg TV, Diener UL (1972) Medium scale production and purification of ochratoxin A, a metabolite of *Aspergillus ochraceus*. Appl Microbiol 23 : 433-435

Davis ND, Searcy JW, Diener UL (1969) Production of ochratoxin A by *Aspergillus ochraceus* in a semi-synthetic medium. Appl Microbiol 17 : 742-744

Diener UL, Davis ND (1966) Aflatoxin production by isolates of *Aspergillus flavus*. Phytopath 56 : 1390-1393

Dubois M, Gills KA, Hamilton JK, Roberts PA; Smith F (1956) In : Methods / Microbiology Norris JR Ribbons DW (eds) Academic Press London pp 272

International Seed Testing Association (1966) International rules for seed testing. Proc Int Seed Assoc 32 : 1-152

Lowry OH, Rosenberough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent J Biol Chem 193 : 265-275

Misra RS, Afsar MR, Singh NK, Singh PL, Sinha KK (1980) Aflatoxin production and loss in calorific value of some seeds infected with *Aspergillus parasiticus*. Nat Acad Sci Letters 3 : 5-6

Moreau C (1979) Moulds, Toxins and Food. Moss Mo (ed) John Wiley and Sons, New York p 477

Nabney J, Nesbitt BF (1965) A spectrophotometric method for determining aflatoxin. Analyst, London 90 : 155-160

Plummer DT (1971) An Introduction to Practical Biochemistry. T.M.H. Publishing Co, Mumbai pp 113-114

Ranjan KS (1985) Histopathological damages in rats by aflatoxin contaminated feed. J Indian Bot Soc 64 : 31-35

Ranjan KS (1986) Survey of different types of animal feeds in relation to aflatoxin elaboration. Bio Bull India 8 : 187-193

Ranjan KS, Sinha AK (1991) Occurrence of mycotoxigenic fungi and mycotoxins in animal feeds of Bihar. India J Sci Food Agric 50 : 39-47

Reddy TV, Vishwanathan L, Venkitasubramanian TA (1970) Thin layer chromatography of aflatoxins. Anal Biochem 38 : 568-571

Roberts BA, Patterson DSP (1975) Detection of twelve mycotoxins in mixed animal feedstuffs, using a novel membrane clean-up procedure. J Assoc Off Anal Chem 58 : 1178-1181

Roe JH, Kuether CH (1943) The determination of ascorbic acid in the whole blood and urine through 2,4-dinitrophenyl hydrazine derivative of dehydro ascorbic acid. J Biol Chem 147 : 399-407

Schwenk E, Alexander GJ, Gold AM. Stevans DF (1958) Biogenesis of citrinin. J Biol Chem 233 : 1211-1213

Scott PM, Lawrence JW, Van Walbeck W (1970) Detection of mycotoxin by thin layer chromatography : Application to screening of fungal extract. Appl Microbiol 19 : 839-842

Singh M, Singh SS, Sanwal GG (1978) A new colorimetric method for the determination of phenolis. Indian J Expl Biol 16 : 712-714

Singh A, Sinha KK (1982) Biochemical changes in musambi fruit inoculated with species of aflatoxin producing *Aspergilli*. Curr Sci 51 : 842-844

Sinha AK, Ranjan KS (1990) Incidence of aflatoxins in foodgrains during flood and post flood period at North Bihar. Proc Indian Natn Sci Acad B56 : 295-298

Sinha KK, Singh A (1982) Aflatoxin producing potentials of *Aspergillus flavus* and natural occurrence of aflatoxins in some fruits and vegetables. Nat Acad Sci Letters 5 : 213-214

Stack KK, Pohland AE (1977) Collaborative study of a method for chemical confirmation of the identity of aflatoxin. J Assoc Off Anal Chem 58 : 110-113

Received 22 March 1999; revised 12 July 2001; accepted 9 December 2001

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 42-46

In vitro and *In vivo* Availability of Iron from *Bathua* (*Chenopodium album*) and Spinach (*Spinacia oleracia*) Leaves

SHASHI KALA YADAV AND SALIL SEHGAL*

Department of Foods and Nutrition,
CCS Haryana Agricultural University,
Hisar - 125 004, India.

Bathua and spinach leaves containing 20.63 and 26.54 mg iron/100 g on dry weight basis were used for estimation of *in vitro* and *in vivo* availability of iron. *In vitro* availabilities of iron were 2.79 and 3.03% of total iron in *bathua* and spinach leaves, respectively. The haemoglobin regeneration efficiency ratios (HRE) of fats fed on *bathua*, spinach and synthetic diet were 19.60, 21.77 and 38.45% respectively. Relative efficiencies of haemoglobin regeneration were 52.31 and 54.44 in *bathua* and spinach fed rats, respectively. The highest apparent iron absorption was observed in the rats fed on synthetic diet and the lowest in rats fed on spinach diet.

Keywords : Apparent iron absorption, *Bathua*, Haemoglobin, *In vitro* and *in vivo* availability, Iron, Relative efficiency, Spinach.

In India, malnutrition is a major nutritional problem, which contributes to about 40-50% of the child deaths (Gopalan et al. 1995). Micronutrient malnutrition poses a serious threat to the health of vulnerable groups of population, particularly the school going children and pregnant women. Though required in small quantities, these nutrients govern many vital functions connected with metabolism, reproduction, immune mechanism and intelligence. These 'micro' nutrients, thus, have 'macro' health implications (Levin et al. 1990).

Iron deficiency anaemia (IDA) is the most prevalent micronutrient deficiency in the world, affecting an estimated 2000 million people both in developed and developing countries (McPhail and Bothwell 1992). IDA is particularly common in the reproductive age group and young children. Iron is an essential element for the formation of haemoglobin (Hb) of red cells and in modulating several cellular functions. Iron deficiency causes a reduction in the number of RBC or the amount of circulating haemoglobin. Hence, iron deficiency status is measured by Hb levels. Multiple factors contribute to IDA, the most important being the inadequate bioavailability of iron in vegetarian whole cereal-based diet (Hallberg et al. 1992). Inadequate iron intakes, low bioavailability and iron loss lead to negative iron balance (Hari Sree and Sharada 1997).

Plant foods are widely consumed and contribute a major percentage of the iron in human diet. Green leafy vegetables are rich sources of iron, but has often been considered a poor source of dietary iron. So, a study was planned to determine the availability of iron *in vitro* and *in vivo* from *bathua* (*Chenopodium album*) and spinach (*Spinacia oleracia*) leaves, as making iron available is an important role of food.

Materials and Methods

Materials : Fresh samples of *bathua* and spinach leaves were obtained from the local market of Hisar city in a single lot, removed healthy leaves and the leaves were washed in running tap water, followed by distilled water and spreaded on rough filter paper sheets to remove excess water, then dried in oven at 60-65^{0}C till dried completely. The dried leaves were powdered in a cyclone mill (mesh size 0.5 mm) individually. The samples were stored in airtight plastic containers for chemical analysis in triplicate and feeding purpose.

Analytical procedures : The proximate composition of the leaves was determined by the methods of AOAC (1990). The total iron contents of the leaves and diet were determined by wet diacid (HNO_3 : $HClO_5$ :: 5:1 v/v) digested samples using Atomic Absorption Spectrophotometer (Lindsey and Norwell 1969). *In vitro* iron availability was determined by the method of Rao and Prabhavathi (1978). Ionizable iron was estimated by AOAC (1990) method.

Diets : The composition of basal diet and experimental diets are given in Tables 1 and 2, respectively. Refined groundnut oil (Ginni brand) was used as source of fat. Freshly purchased $FeSO_4.7H_2O$ was used as reference source of dietary iron. The diets were prepared for one week and kept in refrigerator in airtight plastic containers.

Twenty one days old young albino rats (Wistar strain), weighing 26.41 + 3.23 g were obtained from the Disease Free Small Animal House, College of Veterinary Sciences, CCS Haryana Agricultural University, Hisar. The rats were housed individually in polypropylene cages and kept in air-conditioned room, maintained at 21-22^{0}C with 12 h light and dark cycle.

TABLE 1. COMPOSITION OF BASAL DIET (g/100g DIET)

Ingredients	Amount
Proteins	10.00
Sucrose	10.00
Groundnut oil	10.00
Mineral mixture	4.00
Vitamin mixture	1.00
Cellulose	5.00
DL methionine	0.30
Choline chloride	0.20
Corn starch	To make 100 g

*Corresponding Author

TABLE 2. COMPOSITION OF EXPERIMENTAL DIET (g/kg)

Ingredients	*Bathua* diet	Spinach diet	Synthetic diet
Bathua	150.00	-	-
Spinach	-	113.21	-
Casein	42.70	75.76	117.65
Sucrose	100.00	100.00.	100.00
Cellulose	50.00	50.00	50.00
Vitamin mixture[a]	10.00	10.00	10.00
Mineral mixture[a]	9.52	9.79	40.00
Fat	92.29	94.29	100.00
$FeSO_4.H_2O$	-	-	0.10
DL methionine	3.00	3.00	3.00
Choline tartrate	2.00	2.00	2.00
Corn starch	540.43	541.95	577.35

Note : Casein contained 85.16% protein.

[a] : (AIN - 76A) - based on the National Academy of Sciences recommended level for rats (BARR Committee of Animal Nutrition, 1972)

The rats were made anaemic by feeding the basal diet (Table 1) without adding $FeSO_4.7H_2O$ (11.5 mg iron/kg diet) for 10 days. Approximately, 1ml blood was withdrawn from retroocular capillary on 1st, 7th and 10th days to test their Hb level. The anaemic rats were randomly divided into 3 groups of 9 animals, each balancing across treatments for Hb concentration and body weight.

First group was given *bathua* diet, second group was given spinach diet and third group was given synthetic diet having $FeSO_4.7H_2O$ (0.1 g/kg diet, Table 2). During 20 days of repletion period, each rat was given their respective diet and double distilled water *ad libitum.* Any spilled over diet was weighed and recorded to determine the net diet consumption used for determining iron intake. Fresh diet and double distilled water were given to rat everyday.

Chemical analysis : All the diets were also analysed for their moisture (AOAC 1990) and iron content. Haemoglobin was determined in blood by the method of Oser (1971). The urine, faeces, dried samples of liver, heart, kidney and spleen were wet-digested by the method mentioned above for estimation of total iron content.

The initial and final body weights and haemoglobin levels were measured to calculate Hb regeneration efficiency (HRE) by the following formula given by Mahoney and Hendricks (1982).

Haemoglobin Fe (mg) = W x 0.067 x Hb x 3.35

where W = Body weight (g)

0.067 = ml blood/g body weight (assumed)

Hb = g of haemoglobin/ml blood

3.35 = mg Fe/g haemoglobin (assumed)

$$HRE = \frac{\text{Final haemoglobin Fe (mg)} - \text{Initial haemoglobin Fe (mg)}}{\text{Fe intake (mg)}}$$

Statistical analysis : The data thus obtained were subjected to suitable statistical analysis (Snedecor and Cochran 1967).

Results and Discussion

Proximate composition : The moisture contents of bathua and spinach leaves were 89.04 and 91.05%, respectively (Table 3). When expressed on dry weight basis, the protein, fat, ash, crude fibre contents of *bathua* and spinach leaves were 42.73 and 31.43, 5.14 and 5.05, 20.33 and 26.73, 6.53 and 7.29 g/100 g, respectively.

Iron content of leaves : The iron content of *bathua* and spinach leaves were 20.63 and 26.54 mg/100g, DW, respectively. Higher iron contents in spinach leaves (Gupta et al. 1989; Islam et al. 1987) and in *bathua* leaves (Gopalan et al. 1995) had been reported earlier. On the other hand, Gopalan et al (1995) reported lower iron contents in spinach leaves than the present study. The iron contents of the leaves varied in amounts according to the stage of maturity, conditions of growth, fertilizers used and nature of soil (Bawa and Yadav 1986).

The ionizable iron contents (pH 7.5) of *bathua* and spinach leaves were 4.90 and 5.41% of total iron and their *in vitro* iron availability rates were 2.79 and 3.03% of the total iron, respectively. Chawla et al. (1988) also reported almost similar ionizable iron and its *in vitro* availability in green leaves. They reported that *in vitro* availability of iron from different green leafy vegetables was neither a function of their total iron nor related to their ascorbic acid content but was slightly affected by their oxalic acid content.

In vivo *Iron availability :* Initial body weight of rats fed on *bathua,* spinach and synthetic diets ranged from 25.13 to 27.66 g (Table 4). Feeding the diet containing sub-optimal

TABLE 3. PROXIMATE COMPOSITION, TOTAL IRON AND ITS *IN VITRO* IRON AVAILABILITY IN *BATHUA* AND SPINACH LEAVES (PER 100g, DRY WT. BASIS)*

Parameters	Leaves		SEm±	CD ($P \leq 0.05$)
	Bathua	Spinach		
Moisture, %	89.04 ± 0.09	91.05 ± 0.13	0.27	0.80
Proteins, g	42.73 ± 0.65	31.43 ± 2.10	0.19	0.57
Fat, g	5.14 ± 0.43	5.05 ± 0.40	0.34	NS
Ash, g	20.33 ± 0.34	26.73 ± 1.17	0.28	0.53
Crude fibre, g	6.53 ± 0.21	7.29 ± 0.40	0.14	0.43
Total Iron, mg	20.63 ± 0.24	26.54 ± 0.21	0.30	0.89
Ionizable iron, % of total iron	4.90 ± 0.19	5.41 ± 0.27	0.11	0.34
In vitro available iron, % of total iron	2.79 ± 0.22	3.03 ± 0.31	0.14	0.41

*Values are means ± SD of three independent replicates.

TABLE 4. BIOAVAILABILITY OF IRON FROM BATHUA AND SPINACH IN ANAEMIC RATS*.

Parameters	Iron source			S Em (±)	C.D. (P ≤ 0.05)
	Bathua diet	Spinach diet	Synthetic diet		
No. of rats	9	9	9	-	-
Initial body weight, g	27.66 ± 2.80	26.56 ± 3.66	25.13 ± 2.56	0.47	1.41
Weight of anaemic rats, g	50.88 ± 4.08	51.22 ± 5.07	50.29 ± 2.71	0.66	NS
Final weight, g	57.41 ± 9.26	58.83 ± 6.00	80.22 ± 10.75	1.80	5.42
Body weight gain, g	7.29 ± 4.87	7.44 ± 4.14	30.60 ± 10.53	1.34	4.04
Initial Hb., mg/dl	6.56 ± 0.53	6.88 ± 0.42	6.56 ± 0.53	0.09	0.27
Final Hb., mg/dl	10.51 ± 1.16	10.94 ± 0.83	12.78 ± 1.31	0.20	0.60
Hb. Gain, mg/dl	4.01 ± 0.91	3.94 ± 0.60	6.32 ± 1.16	0.20	0.60
Iron intake, mg	3.06 ± 0.53	2.97 ± 0.34	4.30 ± 0.55	0.23	0.70
H.R.E., %	19.60 ± 4.31	21.77 ± 5.14	38.45 ± 9.14	1.71	5.16
Relative efficiency	52.31 ± 10.88	54.44 ± 12.08	100.00	2.02	6.08

*Values are mean ± SD of nine replicates.

TABLE 5. IRON CONTENT OF DIFFERENT ORGANS OF RATS FED ON DIFFERENT DIETS (MG, ON DRY WEIGHT BASIS)*

Diet	Kidney	Liver	Heart	Spleen
Bathua	0.0295 ± 0.0219	0.3000 ± 0.1529	0.0436 ± 0.01287	0.0458 ± 0.01579
Spinach	0.0252 ± 0.0191	0.2763 ± 0.0429	0.05119 ± 0.01627	0.02730 ± 0.01040
Synthetic	0.0570 ± 0.0193	0.3572 ± 0.0399	0.0556 ± 0.02487	0.05763 ± 0.02463
SEm ±	0.0029	0.0265	0.01225	0.00642
CD (P < 0.05)	0.0087	0.0797	NS	0.01934

*Values are means ± SD of nine replicates, NS : Non significant.

level of iron (11.5 mg/kg diet) for 10 days resulted in significant ($P < 0.05$) reduction of Hb level and during that period, the rats gained body weight significantly. Body weights and Hb levels of anaemic rats ranged from 50.29 to 51.22 g and 6.56 to 6.88 mg/dl, respectively. After 20 days feeding of the rats on experimental diets containing the leafy vegetables and synthetic diet, the final weights varied from 57.41 to 80.22 g. Non-significant ($P < 0.05$) differences in weights were observed in rats fed on *bathua* and spinach diets while weights of rats fed on synthetic diet were significantly higher than those of rats fed on leafy vegetable diets. Weight gains of anaemic rats varied from 7.29 to 30.60 g. Weight gains of rats fed on *bathua* and spinach diet were almost similar, while weight gains of rats fed on synthetic diet were significantly ($P < 0.05$) higher than the rats of the other two groups.

Gains in Hb levels in rats were ranged from 3.94 to 6.32 mg/dl. Gain in haemoglobin level was almost similar in rats fed on *bathua* and spinach diets, while significantly ($P < 0.05$) higher haemoglobin levels were found in rats fed on synthetic diet than the rats fed on *bathua* and spinach diets. Total iron intakes varied from 2.97 to 4.30 mg. Non-significant ($P < 0.05$) differences were observed in iron intakes among rats fed on *bathua* and spinach diets. Iron intakes were significantly higher in rats fed on synthetic diet than rats fed on *bathua* and spinach diets. The haemoglobin regeneration efficiency (HRE) ratios of rats fed on *bathua,* spinach and synthetic diet were 19.60, 21.77 and 38.45%, respectively. HRE was significantly ($P < 0.05$) higher in rats fed on synthetic diet than these fed on *bathua* and spinach diets. Relative efficiency ratios of Hb regeneration ranged from 52.31 to 54.44. The relative efficiency ratio was almost similar in rats fed on *bathua* and spinach diet.

Iron contents of organs (kidney, heart, liver and spleen) were highest in rats fed on synthetic diet, followed by that in rats fed on *bathua* and spinach diet (Table 5). Non-significant differences were observed in iron contents of hearts in rats fed on all three diets.

Mean food intakes were 27.24, 24.32 and 31.37 g in rats fed on *bathua,* spinach and synthetic diets during last five days of the trial, respectively (Table 6). Significant ($P < 0.05$) differences were found in food intakes among rats fed on three different diets.

Mean iron intake was the highest (1.001 mg) in rats and on synthetic diet and the lowest (0.776) in rats fed on spinach diet during metabolic period of study. Iron intakes were significantly different among rats fed on different diets. Excretion of iron (faecal and urinary) was the highest in the rats fed

TABLE 6. FOOD INTAKE, IRON INTAKE, FAECAL AND URINARY IRON EXCRETION AND APPARENT IRON ABSORPTION FROM THE DIFFERENT DIETS*.

Diets	Food intake, g	Iron intake, mg	Faecal & Urinary iron, mg	Apparent iron absorption
Bathua	27.24 ± 6.03	0.850 ± 0.196	0.634 ± 0.221	0.434 ± 0.241
Spinach	24.32 ± 6.20	0.776 ± 0.197	0.672 ± 0.170	0.295 ± 0.092
Synthetic diet	31.37 ± 10.09	1.001 ± 0.332	0.566 ± 0.081	0.449 ± 0.231
SEm ±	1.59	0.018	0.029	0.039
CD ($P \leq 0.05$)	4.80	0.055	0.088	0.117

*Values are means ± SD of nine independent replicates.

on synthetic diet. There were significant differences in the apparent iron absorption among the rats fed on three diets. The highest apparent iron absorption was observed in the rats fed on synthetic diet and the lowest in rats fed on spinach diet. Because of the astringent taste of leaves by virtue of polyphenol and oxalic acid, intake of diet containing these foods may be lower as compared to synthetic diet, which may be free of astringency. Lower intake of any of diet may result in lower intake of minerals, effecting the level of metabolites in blood and organs dependent on the minerals. Zhang et al (1985) and Park et al (1983 a, b) reported that efficiency of converting iron from ferrous sulphate diet into Hb by the anaemic rats was 67%. They also reported that iron of uncooked spinach has been utilized only upto 51% as efficiently as in ferrous sulphate. Campen and Welch (1980) reported that iron deficient rats absorbed 69.7 or 69.8% of the Fe-59 dosed in single meals when given as intrinsically labelled uncooked lypholized spinach or ferric chloride. Gordon and Chao (1984) obtained a relative bioavailability of 75% for spinach as compared to ferrous sulphate using similar methodology. A relative bioavailability of 67% for spinach compared with ferric chloride has been reported by Pye and MaCleod (1946). Reddy and Malewar (1992) reported 3.96% iron bioavailability in spinach. A range of available iron in four amaranth species was narrow, 43-57 ppm (mg/kg) and in feeding trial with rats *Amaranthus tricolor* had 44% available iron (Rangarajan 1997). Thus, iron-bioavailability as determined in this study appears to be lower than previously reported by others.

Conclusion

It may be concluded from this study that iron contents of these green leafy vegetables varied from 20 to 26% and *in vitro* availabilities of iron ranged from 2.79 to 3.03%. The leaves helped in increasing the haemoglobin level though relative efficiency of haemoglobin regeneration efficiency was 52.31 to 54.44 which was significantly lower than the synthetic diet but still it could provide good haemoglobin regeneration efficiency. Therefore, it is recommended that these green leafy vegetables should be included in the diets of general masses especially adolescent girls, who are prone to iron deficiency anaemia. Nutrition education programme should also emphasize the use of green leafy vegetables, which are inexpensive and can be used easily by community.

References

AOAC (1990) Official Methods of Analysis, 14th edn. Association of Official Agricultural Chemists, Washington, DC

Bawa SF, Yadav SP (1986) Protein and mineral contents of green leafy vegetables consumed by Sokoto population. J Sci Food Agric 37 : 504-506

Campen DR van, Welch RM (1980) Availability to rats of iron from spinach : Effect of oxalic acid. J Nutr 110 : 1618-1621

Chawla S, Saxena A, Seshadri S (1988) *In vitro* availability of iron in various green leafy vegetables. J Sci Food Agric 46 : 125-127

Gopalan C, Ramasastri BV, Balasubramanian SC, Narasingha Rao B, Deosthale YG, Pant KC (1989) Nutritive Value of Indian Foods. National Institute of Nutrition, ICMR, Hyderabad, India. pp 60-61

Gorden DT, Chao LS (1984) Relationship of components in wheat bran and spinach to iron bioavailability in the anemic rat. J Nutr 114 : 526-535

Gupta K, Barat GK, Wagle DS, Chawla HKL (1989) Nutrient contents and antinutritional factors in conventional and non-conventional leafy vegetables. Food Chem 31 : 105-116

Hullberg L, Rossander Hulten L, Brune M, Gleerup A, Sandberg AS (1992) Iron absorption from bread : Inhibitorying effect of cereal fibre phytate and inositol phosphates with different numbers of phosphate groups. J Nutr 122 : 442-449

Hari Sree T, Sharada D (1997) Iron nutritional status and work performance. Food Nutr News 2(1) : 1-2

Islam MN, Genurrio RR, Pappas-Sirois M (1987) Nutritional and sensory evaluation of *Atriplex triangularis* leaves. Food Chem 25 : 279-284

Levin HE, Pollitt R, Galloway, McGuire JM (1990) Micronutrient Deficiency Disorders. Draft Monogrpah. World Bank Health Sector Priority Review, Washington DC

Lindsey WL, Norwell MA (1969) A new DPTA-TEA soil test for zinc and iron. Agron Abstr 61 : 84

Mahoney AW, Hendricks DG (1982) Efficiency of haemoglobin regeneration as a method of assessing iron bioavailability in food products. In : Nutritional Bioavailability of iron. Kies c (ed) Am Chem Soc, Washington DC

McPhail P, Bothwell TH (1992) The prevalence and causes of nutritional iron deficiency. In : Nutritional Anaemias : Fomon Z (ed) Raven Press, New York, pp 1-3

Oser BL (1971) Hawk's Physiological Chemistry. 15th edn. McGraw Hill Inc, New York

Park YW, Mahoney AW, Cornforth DC, Collenge SK, Hendricks DG (1983 a) Bioavailability to anaemic rats of iron from fresh cooked of nitrosylated haemoglobin and myoglobin. J Nutr 173 : 680-

Park YW, Mahoney AW, Hendricks Dg (1983 b) Bioavailability of different sources of ferrous sulphate iron red to anaemic rats. J Nutr 113 : 2223-2228

Pye OF, Macleod G (1946) The utilization of iron from different foods by normal young rats. J Nutr 32 : 667-672

Rangarajan A (1997) Exploring the potential to enhance dietary iron from a green leafy vegetable In. Agricultural Production and Reply to the Querries Nutrition Lockeretz W (ed). Proceedings of an International Conference, Boston, Massachusetts, USA, 19-21 March

Rao BSN, Prabhavathi T (1978) An *in vitro* method for predicting the bioavailability of iron from foods. Am J Clinc Nutr 31 : 169-175

Reddy NS, Malewar VG (1992) Bioavailability of iron from spinach (*Spinacia oleracia*) cultivated in soil fortified with graded level of iron. Plant Food Human Nutr 42(4) : 313-318

Snedecor GW, Cochran WG (1967) Statistical Methods. State University Press, Ames, Iowa, USA

Zhang D, Hendricks DG, Mahoney AW, Cornforth DP (1985) Bioavailability of iron in green peas, spinach, cereal bran and corn meal fed to anaemic rats. J Food Sci 50(2) : 426-431

Received 31 August 2000; revised 13 July 2001, accepted 9 December 2001

RESEARCH PAPER

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 47-52

Studies on the Effect of Sterols on Ethanol Production

A.B. CHAUDHARI, S.K. TALEGAONKAR AND S.B. CHINCHOLKAR*

Department of Microbiology,
School of Life Sciences,
North Maharashtra University,
P.B. No. 80, Jalgaon-425 001, India.

Schizosaccharomyces Y11 isolate and reference yeast strain were used for the production of ethanol at 27°C, pH 4.5 in high gravity (20% DS) molasses medium fortified with sterols and ternary complex (sterol-Tween 80-albumin). While sterols alone were ineffective in converting sugar to ethanol as compared to unsupplemented worts, incorporation of ternary complex in high gravity wort showed 95% sugar utilization with improved (above 90%) fermentation efficiency in all yeast strains. *Schizosaccharomyces* Y11 isolate produced 70 gL^{-1} ethanol in cholesterol- and stigmasterol-ternary complex supplemented worts. Higher ethanol yield (86.4 gL^{-1}) was obtained from stigmasterol-Tween 80-albumin (ternary complex) supplemented high gravity wort (27% DS) presumably due to increased viability of yeast strains.

Keywords : Sterols, Ternary complex, *Schizosaccharomyces* pombe, *S. cerevisiae,* Ethanol production.

Alcoholic fermentation received worldwide attention as an efficient alternative energy source since several decades. Attempts to increase ethanol concentration during fermentation adversely affected growth, viability and fermentation rate of yeast cells (Van Uden 1984; Chaudhari and Chincholkar 1996). This toxicity has been found to be associated with protein denaturation, membrane fluidity (Koukou et al. 1993), adverse effect on membrane associated enzymes (Lloyd et al. 1993; Alexandre et al. 1994, 1996), leading to membrane leakage, yeast viability, fermentation capacity (Ohata and Hayashida 1983; Mauricio et al. 1991) and promoting mitochondrial DNA mutagenesis (Piper 1995). However, high ethanol tolerance is a pre-requisite for achieving higher ethanol yield (del Castillo Agudo 1992). Therefore, improvement in the yield of ethanol by exogenous supplementation of various metabolites in the growth medium and tolerance of yeast to ethanol has attracted considerable attention. Supplementation includes addition of lipids, proteins, vitamins (Casey et al. 1983; Mizoguchi and Hara 1997), due to their significant role in maintaining membrane fluidity and influencing activity of membrane-associated enzymes (Lloyd et al. 1993; Alexandre et al. 1994, 1996) by virtue of sterols and fatty acids being the major components of cytoplasmic membrane of cells.

In the present paper, plasma membrane being sensitive to ethanol, influence of sterols and ternary complex supplementation on ethanol production were examined in order to derive a relationship with viability and their ability to tolerate alcohol inhibition.

Materials and Methods

Microorganisms : Reference yeast cultures, *Saccharomyces cerevisiae* NCIM 3085, *Saccharomyces uvarum* NCIM 3455 and *Schizosaccharomyces* pombe NCIM 3457 were obtained from National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India. The osmotolerant yeast isolate used in this study was obtained from distillery rivulet, identified as *Schizosaccharomyces* Y11 as reported earlier (Chaudhari and Chincholkar 1999).

*Corresponding Author E-mail: chincholkar_sb@rediffmail.com

These cultures were maintained on YM agar slants. Cultures were acclimatized for sugarcane molasses fermentation by growing in a MYPM growth medium containing increasing concentration of molasses and finally in MYPM growth medium comprising of ($g L^{-1}$) : malt extract, 3; yeast extract, 3; peptone, 5 and molasses total sugars (M) in glucose equivalent, 150.

Culture medium and fermentation conditions : Biomass required for batch fermentation was obtained by cultivating yeast strains in shake flasks on a rotary shaker at 180 rpm and 27°C according to the method described by Hsiao et al (1983). Actively growing (18 h) cells from logarithmic phase were harvested by centrifugation, washed thrice and pre-treated to avoid contamination during fermentation (Arbatti and Kale 1990). The fermentation medium was inoculated with 40 gL^{-1} inoculum on wet weight basis, grown for 24 h under aerobic conditions. After inoculation, batch fermentations were conducted in 150 ml flasks containing 100 ml fermentation medium comprising 150 gL^{-1} and 200 gL^{-1} in total sugar molasses with 0.1% urea (pH 4.5) at 27°C under static condition. Aliquots were removed for determining ethanol and cell viability at finite intervals. All experiments were run in triplicate and values reported are averages.

Sterol supplement : To assess the effect of supplementation of sterols on fermentation, optimal concentration of sterols (cholesterol, sitosterol and stigmasterol) with Tween-80, cost-effective adjunct to sterols (pressmud extract) and Tween 80 and sterol-albumin-Tween 80 complex (ternary complex) was studied with Y11 and reference strains in high gravity (150, 200 g L^{-1} glucose equivalent of fermentable sugars) molasses medium for ethanol production according to the method described by Ohata and Hayashida (1983). A control experiment without supplementing fermentation medium was simultaneously carried out under identical conditions (Chaudhari and Chincholkar 1999).

For this purpose, ternary complex was prepared by mixing 0.5 mg Tween 80 + 1.2 mM sterol and 5 mg albumin per ml, shaking vigorously in a sterile culture bottle and used as a supplement in molasses medium. Similarly, pressmud extract, obtained by using benzene soxhlet extraction, was also

explored for the same purpose.

Determination of parameters : Percent viable yeast cells in fermentation wort was determined under a light microscope using a Neubaur counting chamber (Lee et al. 1981).

Residual sugars in the fermentation wort were determined after inversion with HCl using DNSA method (Miller 1959), whereas, ethanol was quantified after distillation by using ferric ammonium nitrate method (Reid and Truelove 1952).

Results and Discussion

Influence of sterol supplements on ethanol production : In conventional commercial processes, worts of 12-18% dissolved solids (DS) are generally fermented to produce ethanol. Attempts to ferment worts above 18% DS by supplementation with various metabolites for higher ethanol concentration have attracted worldwide attention largely because of reduced yeast viability of cells, leading to slow and incomplete fermentation. It has been reported that there was a drastic fall in the rate of fermentation, especially in a continuous system, with three-residence interval. Used cells had to be continually replenished or intermittently discarded completely. Patil and Patil (1990a, b) also observed similar effect upon supplementation of fungal mycelium in fermentation wort for enhanced ethanol production.

In high gravity worts, oxygen availability diminished due to decreasing solubility of oxygen with increasing DS. Oxygen is essential in yeasts for the synthesis of sterols and unsaturated fatty acids, (Mauricio et al. 1998). Oleic acid-rich yeast membrane prompted Thomas and Rose (1979) to postulate its enhanced tolerance to ethanol. Both, sterols and unsaturated fatty acids were correlated to an activated growth and promoted yeast survival in the high gravity wort's fermentation (Moulin et al. 1984). In fact, ethanol tolerance of certain yeasts depended on sterol and unsaturated fatty acids composition of their plasma membrane (Mishra and Prasad 1989; Koukou et al. 1993; Baleiras and Huis 1995). They imparted 'fluidity' to plasma membrane. Since their synthesis did not occur under anaerobic conditions, idea of supplementation of sterols and unsaturated fatty acids to relieve yeast cells from ethanol toxicity attracted considerable attention. Perhaps this was reflected from the observation of Bajpai et al (1998) that supplementation of fermentation wort with soy flour increased the rate of fermentation and final ethanol concentration. Li (1995) also could improve ethanol yield by incorporating soyflour in inoculum medium rather than fermentation medium.

Andreasen and Stier (1954) used ergosterol, oleic acid and Tween 80 in an anaerobic shake culture of *S. cerevisiae* in a chemically defined medium. Ohata and Hayashida (1983) also worked on this line. Whether physiology would respond in a similar manner in Y11 isolate, was explored in the present investigation by studying the effect of sterols, sterol-substitute, Tween 80 and protein.

Effect of sterol plus Tween 80 on ethanol production : In high gravity (20 and 27% DS) fermentation medium (150 and 200 gL^{-1} total reducing sugars), supplemented with sterols (stigmasterol/ cholesterol/ sitosterol) and Tween 80, a series of experiments was carried out at pH 4.5, 27°C under standardized conditions for isolate Y11. The rates of sugar uptake and ethanol productivity were monitored. Table 1 gives fermentation profiles of isolate Y11 and reference yeast strains.

TABLE 1. EFFECT OF STEROL PLUS TWEEN 80 ON FERMENTATIVE EFFICIENCY OF YEAST STRAINS IN MOLASSES MEDIUM CONTAINING 150 gL^{-1} GLUCOSE EQUIVALENT OF TOTAL SUGARS AT 27°C

Yeast strain	Sterol supplement	Sugar utilization, %	Ethanol, gL^{-1}	Ethanol yield, gg^{-1}	Fermentation efficiency, %
Schiz.	A	93	59.0	0.422	83
pombe	B	96	59.0	0.410	80
Y11	D	92	55.0	0.405	79
	F	93	62.8	0.449	88
	None	97	66.5	0.450	89
S. cerevisiae	A	92	55.4	0.405	79
NCIM	B	93	59.0	0.422	83
3085	D	90	51.7	0.380	74
	F	95	62.8	0.422	83
	None	99	62.8	0.412	81
Schiz.	A	92	51.7	0.375	74
pombe	B	97	55.4	0.380	74
NCIM	D	92	55.4	0.401	79
3457	F	96	62.7	0.462	90
	None	99	59.0	0.405	79

A : cholesterol; B : cholesterol oleate; D : sitosterol; F : stigmasterol

Supplement of sterol (1.2 mM stigmasterol / sitosterol / cholesterol); 1.2 mM cholesterol oleate, Tween 80 (0.5 mg/ml) and an initial cell density of 40 gL^{-1} was used. Percentage of theoretical yield of ethanol was calculated on the basis of sugar consumption.

Data presented in Table 1 show that regardless of the strain, (i) sterols alone (cholesterol, sitosterol or stigmasterol) were ineffective in improving sugar utilization and (ii) cholesterol oleate also did not improve the performance as judged by % fermentation efficiency. The role of Tween 80 was presumably that of their solubilizer, necessary for entry through membrane. These results have stressed that sterols alone or their oleates were ineffective.

Reproducibility of effect of sterols-albumin-Tween 80 (ternary) complex on ethanolic fermentation : The above experiments were repeated after the incorporation of albumin into sterol-Tween 80 mixture to form sterol-Tween-80-albumin (ternary) complex (Table 2). It was observed that as a result of sterol-Tween 80-albumin complex (from either of three sterols) : (i) the rate of sugar utilization by all the yeast strains at the end of 34 h was above 95%, (ii) fermentation efficiency in all the yeast strains was above 90%, (iii) among the sterol combinations, stigmasterol-Tween 80-albumin complex gave maximum (93%) fermentation efficiency, (iv) CO_2 formation increased remarkably, corroborating observation in (i) and (ii). (v) final ethanol concentration by Y11 isolate was 70 gL^{-1} in the presence of cholesterol- and stigmasterol- ternary complex.

TABLE 2. EFFECT OF STEROL PLUS TWEEN 80 AS ALBUMIN (TERNARY COMPLEX) ON FERMENTATIVE EFFICIENCY OF YEAST STRAINS IN HIGH GRAVITY MOLASSES MEDIUM CONTAINING 150 gL^{-1} GLUCOSE EQUIVALENT OF TOTAL SUGARS AT 27°C

Yeast strain	Sterol supplement	Sugar utilization, %	Ethanol, gL^{-1}	Ethanol yield, gg^{-1}	Fermentation efficiency, %
Schiz.	C	99	66.8	0.463	91
pombe	E	97	62.8	0.430	84
Y11	G	99	70.0	0.474	93
	None	97	66.5	0.450	89
S.	C	97	66.7	0.462	91
cerevisiae	E	97	66.5	0.455	89
NCIM	G	99	70.0	0.473	93
3085	None	99	62.8	0.412	81
Schiz.	C	97	66.5	0.456	89
pombe	E	96	62.7	0.430	84
NCIM	G	100	66.7	0.467	92
3457	None	99	59.0	0.405	79

C : cholesterol + Tween 80 + albumin; E : sitosterol + Tween 80 + albumin; G : stigmasterol + Tween 80 + albumin.

Supplement of sterol (1.2 mM stigmasterol / sitosterol / cholesterol); Tween 80 (0.5 mg/ml) and albumin (5 mg/ml) and an initial cell density of 40 gL^{-1} was used as inoculum. Percentage of theoretical yield of ethanol was calculated on the basis of sugar consumption.

TABLE 3. INFLUENCE OF STEROL SUPPLEMENTATION ON UTILIZATION OF SUGAR AND PRODUCTION OF ETHANOL BY YEAST STRAINS IN MOLASSES (150 gL^{-1} TOTAL SUGARS) AT 27°C AND pH 4.5

Sterol supplements	Sugar, gL^{-1} Sample withdrawn, h				Ethanol, gL^{-1} Sample withdrawn, h			
	5	15	24	34	5	15	24	34
***Schizosaccharomyces* Y11**								
A	106	86	50	10	11.0	33.2	44.3	59.0
B	94	70	26	06	18.4	44.3	51.7	59.0
C	86	58	14	02	25.2	48.0	66.5	66.7
D	116	90	48	12	11.0	31.0	45.0	55.4
E	88	64	14	04	14.8	43.8	59.0	62.8
F	110	84	30	10	14.8	43.8	50.0	62.8
G	80	52	08	02	25.9	51.7	66.7	70.1
H	96	56	16	04	18.4	51.7	55.1	62.8
None (control)	94	54	14	04	22.1	51.7	62.8	66.5
***S. cerevisiae* NCIM 3085**								
A	110	84	40	12	14.8	29.5	43.8	55.4
B	96	66	22	10	14.8	33.2	55.4	59.1
C	90	54	12	04	18.4	48.0	62.8	66.1
D	120	70	38	14	14.8	33.2	48.0	51.7
E	88	62	10	04	14.8	43.8	62.8	66.5
F	118	68	18	08	18.5	36.9	51.7	62.8
G	86	50	06	02	22.2	51.7	66.7	70.1
H	100	48	18	02	22.2	36.9	59.1	62.8
None (control)	100	60	18	04	22.2	40.6	59.1	62.8
***Schizosaccharomyces pombe* NCIM 3457**								
A	110	84	42	12	11.0	29.5	48.0	51.7
B	94	70	28	04	14.8	33.2	51.7	55.4
C	88	54	12	04	18.4	44.3	62.8	66.5
D	118	86	40	12	14.8	36.9	48.0	55.4
E	94	64	12	06	11.0	48.0	51.7	62.7
F	118	80	16	06	11.1	36.9	48.0	66.7
G	84	50	06	00	22.2	40.6	70.1	70.1
H	90	52	10	04	22.1	40.6	48.0	62.8
None (control)	100	64	14	06	22.1	40.6	59.1	59.6

A-cholesterol; B-cholesterol oleate; C-cholesterol-ternary complex; D-sitosterol; E-siterol-ternary complex; F-stigmasterol; G-stigmasterol-ternary complex; H-pressmud extract. Inocula were raised at 25°C and an initial cell density of 40 gL^{-1} was used.

in just 24 h, (vi) the reference strain produced 66.5 and 70 gL^{-1} ethanol with cholesterol- and stigmasterol- ternary complexes, respectively, (vii) *S.cerevisiae* produced 66.7 gL^{-1} ethanol as a function of cholesterol, stigmasterol- and sitosterol- ternary complex supplements, (viii) viability of isolate Y11 was 90% in stigmasterol ternary complex-enriched fermentation wort, higher than unsupplemented (control) wort and (ix) reference strains showed 84-85% viability. These results are shown in Table 3 depicting profiles of sugar consumption and depicting profiles of ethanol production. The above results, corroborated each other, to show that ternary complexes improved permeability due to Tween-80, thereby exerting maximum effect in consonance with the use of soyflour, as a natural complex of sterols and protein. Finally, reproducibility of the results confirmed that these observations were not a coincidence, but a scientific reality. These results were in accordance with those observed by Mauricio et al (1998).

Supplementation of sterols as practised in the above experiments does neither appear to be economically viable, nor logistically feasible on a sustained basis. So, an effective way was to supplement an economically feasible alternative, which made scientific and logistic sense. For this purpose, while soyflour or pressmud could be employed, as a rich source of sterols. Among these two, soyflour was costly (Rs. 25,000/tonne) and its effect was already explored by Li (1995), while pressmud is cheap (Rs. 150/tonne), abundantly available and possessed complex composition to-meet contingencies not explored hethereto. Hence in this study, use of crude wax derived from pressmud (contains 7-9% sterols out of which 20"% is stigmasterol and 80% sitosterol) was examined in a high gravity fermentation wort for ethanol production. In spite of incorporation of cost-effective adjunct to sterols (pressmud extract) in high gravity worts, there was no productivity. It is not clear at this stage if (a) added amount was sub-optimal, (b) strain Y11 was unresponsive to addition, and (c) other

TABLE 4. INFLUENCE OF STIGMASTEROL TERNARY COMPLEX ON OVERALL KINETIC PARAMETERS OF HIGH GRAVITY MOLASSES FERMENTATION TO ETHANOL BY YEASTS AT 27°C

Yeast strain	Sterol in wort	Initial sugar, gL^{-1}	Residual sugar, gL^{-1}	Max. ethanol, gL^{-1}	Sugar utilization, %	Ethanol yield, gg^{-1}	Theoretical yield, %
Schiz Y11	A	150	04	66.5	97.0	0.456	89
	B	150	10	62.8	93.0	0.449	88
	C	150	02	70.1	98.6	0.473	93
	A	200	20	74.4	90.0	0.413	80
	B	200	14	74.4	93.0	0.400	78
	C	200	08	86.4	96.0	0.449	88
S. pombe	A	150	02	59.1	98.6	0.399	78
NCIM	B	150	06	66.5	96.0	0.416	90
3457	C	150	02	70.1	98.6	0.474	93
	A	200	18	74.4	91.0	0.408	79
	B	200	18	74.4	91.0	0.408	79
	C	200	10	78.4	95.0	0.413	81

A : control; B : stigmasterol; C : ternary complex (Stigmasterol-Tween-80-albumin) Inocula were raised at 27°C and an initial cell density of 40 gL^{-1} was used. Percentage of theoretical yield of ethanol was calculated on the basis of sugar consumption.

TABLE 5. EFFECT OF STEROL SUPPLEMENTATION ON SUGAR CONSUMPTION AND ETHANOL PRODUCTION BY *Schizosaccharomyces* Y11 IN 200 gL^{-1} SUGAR CONTAINING MOLASSES WORTS AT 27°C, AND pH 4.5

Sterol supplements	Sugar, gL^{-1}						Ethanol, gL^{-1}					
	Sample withdrawn, h						Sample withdrawn, h					
	5	15	20	25	30	35	5	15	20	25	30	35
A	132	112	94	56	30	20	19.6	35.2	54.8	62.7	74.4	74.4
B	144	126	106	68	42	14	18.4	40.0	47.0	58.7	62.7	74.4
C	128	106	84	50	26	06	22.0	48.0	58.7	70.5	78.4	86.2
D	138	114	102	68	44	18	18.4	36.9	47.0	51.7	62.7	74.4
E	132	108	98	58	40	12	22.1	44.3	54.8	62.7	70.5	78.4

A-control; B-stigmasterol oleate; C-stigmasterol ternary complex; D-cholesterol Oleate; E-cholesterol ternary complex. Inocula were raised at 27°C and an initial cell density of 40 gL^{-1} was used.

ingredients of pressmud are counter-productive.

Effect of stigmasterol ternary complex on yet higher gravity molasses fermentation : The influence of stigmasterol ternary complex on isolate Y11 and *Schiz.* pombe NCIM 3457 was further investigated in an yet high (200 g L^{-1} total reducing sugars) gravity molasses medium at 27°C. The data summarized in Tables 4 and 5 show that (i) the ternary complex was more effective *vis-a-vis* mere stigmasterol/ control, (ii) 150 g L^{-1} sugar gravity gave the higher yield of ethanol over 200 g L^{-1} sugar gravity medium, (iii) process efficiency as judged from % theoretical yield was higher with 150 g L^{-1} medium *vis-a-vis* 200 g L^{-1} medium, (iv) isolate Y11 was superior to reference strain (v) effect of cholesterol tertiary complex was inferior to stigmasterol ternary complex and (vi) as depicted in Table 6, maximum viability (89%) was observed in stigmasterol ternary complex containing high gravity wort after 35 h.

Studies by Casey et al (1983, 1984), Walker-Caprioglio et al (1990) and Swan and Watson (1997) have shown that the action of ethanol on yeast was influenced by sterol (ergosterol) and lipid composition of plasma membrane. The addition of Tween 80 plus sterol (especially stigmasterol) to the high gravity wort as albumin complex made it possible to obtain higher concentrations of ethanol than when free sterol or pressmud extract was used. For distillery yeasts, to produce high levels of ethanol efficiently, the wort must contain nitrogen source (provided in this study by albumin), a combination of sterol (provided in this nitrogen source (provided in this study by albumin), a combination of sterol (provided in this work by cholesterol/ sitosterol/ stigmasterol) and unsaturated fatty acid (provided as oleic acid fraction of Tween 80).

Casey et al (1983) emphasized that both supplements (protein and ternary complex) were simultaneously needed for rapid anaerobic fermentation of high gravity worts. These supplements promoted cell mass synthesis, increasing the

TABLE 6. EFFECT OF STEROL SUPPLEMENTATION ON VIABILITY OF *Schizosaccharomyces* Y11 IN HIGH GRAVITY WORTS (200 gL^{-1} TOTAL SUGAR) AT 27°C, pH 4.5

Sterol supplements	Viability, %		Cell count, X 10^8	
	Sample withdrawn, h		Sample withdrawn, h	
	15	35	15	35
A	87	70	3.05	2.15
B	89	85	3.70	2.97
C	92	89	4.60	4.01
D	88	80	3.87	3.68
E	90	80	4.13	4.07

A-control; B-stigmasterol oleate; C-stigmasterol ternary complex; D-cholesterol Oleate; E-cholesterol ternary complex

population of new yeast and improving their viability.

The current practice in distillery is the use of high gravity wort so as to reduce the cost of product distillation and recovery. For doing so, sterols, lipids and nitrogen deficiencies need to be overcome to enhance the rate of ethanol production. This is especially true, since lipids are present in growth limiting concentration, even in normal gravity worts (David and Kirsop 1972). Therefore, they must be synthesized by yeasts during the first phase of fermentation, when oxygen is still available (Kirsop 1982). However, solubility of oxygen decreases in high gravity worts, which, if unsupplemented, will certainly have oxygen as the growth limiting vital necessity. As demonstrated in this investigation, oxygen limitation can be partially alleviated by the addition of Tween 80-stigmasterol-albumin in high gravity fermentation medium (200 gL^{-1} total fermentable sugars). The albumin in ternary complex (5 mg/ml) acted as a source of nitrogen in the beginning, enough to cause yeast growth for which Casey et al (1984) incorporated free amino nitrogen.

It is apparent that high gravity fermentation increases ethanol yield, eventually from non-proportional increase in cell mass (Palmer and Rennie 1974). Presently, such approach is inappropriate because commercial worts are of 16-18% DS. Such processes warrant modification(s) for (i) use of higher levels of cheaper adjuncts, (ii) sustained availability of oxygen, (iii) sustained replenishment of active cells, and (iv) medium composition permitting fluidity in yeast membrane, which in turn, neutralised stressful effect of high gravity, high temperature and high ethanol content, ultimately leading to efficient fermentation.

In conclusion, the inhibitory effect of ethanol under anaerobic condition on cell vitality and fermentation activity of yeast isolate and reference strains can be compensated by adding ternary complex to the high gravity fermentation wort.

Acknowledgement

This work was supported by University Grants Commission, New Delhi (India) under the 8th five year plan of teacher fellowship. The authors thank Prof. R.M. Kothari, Director, School of Life Sciences for critical evaluation of this paper.

References

Alexandre H, Rousseaux I, Charpentier C (1994) Relationship between ethanol tolerance, lipid composition and plasma membrane fluidity in *S. cerevisiae* and *K. apiculata*. FEMS Microbiol Lett 124: 17-22

Alexandre H, Mathieu B, Charpentier C (1996) Alteration in membrane fluidity and lipid composition and modulation of H^+ - ATPase activity in *S. cerevisiae* caused by decanoic acid. Microbiol 142; 469-475

Andreasen AA, Stier TJB (1954) Anaerobic nutrition of *Saccharomyces II* : Unsaturated fatty acids requirement for growth in a defined medium. J Cellular Comparative Physiol 43: 271-281

Arbatti SV Kale UM (1990) Scope for improvement in conventional batch process. Bhartiya Sugar 15:33-36

Bajpai P, Sharma A, Raghuram N, Bajpai PK (1998) Rapid production of ethanol in high concentration by immobilized cells of *S. cerevisiae* through soyflour supplementation. Biotechnol Bioengg 10:217-220

Baleiras Couto MM, Huis in't Veld JH (1995) Influence of ethanol and temperature on the cellular fatty acid composition of *Z. bailii* spoilage yeasts. J Appl Bacteriol 78:327-334

Casey GP, Magnus CA, Ingledew WM (1983) High gravity brewing: nutrition enhanced production of high concentration of ethanol by brewing yeast. Biotechnol Lett 5:429-434

Casey GP, Magnus CA, Ingledew WM (1984) High gravity brewing: effects of nutrition on yeast composition, fermentative ability and alcohol production. Appl Environ Microbiol 48:639-646

del Castillo Agudo L (1992) Lipid content of *S. cerevisiae* strain with different degrees of ethanol tolerance. Appl Microbiol Biotechnol 37:647-651

Chaudhari AB, Chincholkar SB (1996) Cell immobilization. A critical approach to ethanol production by *S. cerevisiae* and *Schizosaccharomyces* pombe. Indian J Microbiol 36:75-83

Chaudhari AB, Chincholkar SB (1999) New osmotolerant *Schizosaccharomyces* for ethanol production. J Food Sci Technol 36:166-169

Espionsa R, Cojulun V, Manroquin F (1978) Alternatives for energy savings at plant levels for production of alcohol for the use of automotive fuel. Biotechnol Bioengg Symp 8:69-74

Hsiao H, Chiang L, Yang C (1983) Preparation and performance of immobilized yeast cells in columns containing no inert carrier. Biotechnol Bioengng 25:363-375

Koukou AI, Tsoukatos D, Drainas C (1993) Effect of ethanol on the sterols of the fission yeast *Schizosaccharomyces pombe*. FEMS Microbiol Lett 111 : 171-177

Lee SS, Robinson FM, Wang HY (1981) Rapid determination of yeast viability. Biotechnol Bioengng Symp 11 : 641-649

Lloyd D, Morrell S, Carlsen HN, James PE, Rowlands CC (1993) Effect of growth with ethanol on fermentation and membrane fluidity of *S. cerevisiae*. World J Microbiol Biotechnol 9 : 825-833

Li X (1995) Improvement in ethanol production from beet molasses by soyflour supplementation. Biotechnol Letts 17 : 327-330

Mauricio JC, Guijo S, Ortega JM (1991) Relationship between the phospholipid and sterol contents in *S. cerevisiae* and *T. delbrueckii* and their fermentation activity in grape musts. American J Enol Vitic 42 : 301-308

Mauricio JC, Millan C, Ortega JM (1998) Influence of oxygen on the biosynthesis of cellular fatty acids, sterols and phospholipids during alcoholic fermentation by *S. cerevisiae & T. delbrueckii.* World J Microbiol Biotechnol 14 : 405-410

Miller GL (1959) Use of dinitrosalicylic acid (DNSA) reagent for determination of reducing sugars. Analyt Chem 31 : 426-428

Mishra D, Prasad R (1989) Relationship between ethanol tolerance and fatty acyl composition of *S. cerevisiae.* Appl Microbiol Biotechnol 30 : 294-298

Mizoguchi H, Hara S (1997) Ethanol induced alterations in lipid composition of *S. cerevisiae* in presence of exogenous fatty acid. J Ferment Biotechnol 83 : 12-17

Moulin G, Boze H, Galzy P (1984) Inhibition of alcoholic fermentation. In : Biotechnology Genetic Engineering Review, Russell GE (ed.), Interscept, Newcastle, pp. 365-384

Ohata K, Hayashida S (1983) Role of Tween 80 & monoolein in a lipid - sterol-protein complex which enhances ethanol tolerance of sake yeast. Appl Environ Microbiol 46 : 821-825

Palmer AK, Rennie H (1974) Ester control in high gravity brewing. J Inst Brew 80 : 447-454

Patil SG, Patil BG (1990 a) Acceleration of ethanol production activity of yeast in cane molasses fermentation by the addition of fungal mycelium. Enzyme Microbial Technol 12 : 141-148

Patil SG, Patil BG (1990 b) Binary yeast cultures in presence of supplements enhance the rate of ethanol production in cane molasses. Bhartiya Sugar 15 : 37-40

Piper PW (1995) The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. FEMS Microbiol Letts 134 : 121-127

Reid VW, Truelove RK (1952) The colorimetric determination of alcohol. Analyst 77 : 325-328

Thomas DS, Rose AH (1979) Inhibitory effect of ethanol on growth & solute accumulation by *S. cerevisiae* as effected by plasma membrane lipid composition. Arch Microbiol 122 : 49-55

Van Uden N (1984) Effects of ethanol on the temperature relations of viability and growth in yeasts. Crit Rev Biotechnol. 1 : 263-272

Received 7 March 2000; revised 16 July 2001; accepted 10 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 53-54

Effect of Pre-Harvest Chemical Treatments On Shelf Life of Pineapple Fruit

S. TALUKDAR AND F. AHMED*

Department of Crop Physiology,
Assam Agricultural University,
Jorhat - 785 013, India.

Maturing kew pineapple fruits were sprayed with calcium chloride (1 and 2%), sodium benzoate (0.25 and 0.5%), cycocel (0.05 and 0.1%) and gibberellic acid (0.01 and 0.02%). After harvesting, physiological weight loss, sugars and titrable acidity were analysed *vis-a-vis* the shelf life at ambient temperature. Calcium chloride (2%) was found to be the most effective pre-harvest treatment.

Keywords : Kew pineapple, Shelf life, Pre-harvest chemical treatment.

Pineapple is produced abundantly in the North-East region of India. Being a fleshy and juicy fruit, its shelf life is very short. A considerable portion of this crop is damaged before reaching the consumers and processing units. The average post-harvest loss is estimated to be 21 % but occasional instances may go upto 45 % (Anon 1978). Depending on maturity at harvest and storage condition, post-harvest life is further reduced due to a number of disorders, diseases, microbial spoilage etc (Akamine and Goo 1963). These situations necessitate the extension of storage life of pineapple fruit over an extended period. Foliar applications of some chemicals like calcium chloride, sodium benzoate, gibberellic acid, cycocel etc. are increasingly used now-a-days (Bhangia and Lenka 1994; Khader 1991; Misra et al. 1978) to extend the shelf life of different fruits. The present communication deals with studies on the role of some chemicals in extending the storage life of pineapple fruits.

The present investigation was carried out at Assam Agricultural University, Jorhat, India. The variety selected was a kew pineapple from a three years old ratoon pineapple orchard. To enhance and bring about uniform flowering ethrel (25 ppm) alongwith 2% urea and 0.04% calcium carbonate was sprayed on the heart of each plant just before the natural flowering time (March/April). Flowering was recorded and the developing fruits were labelled. The experiment was laid out in a randomized block design with three replications. There were altogether nine treatments including water spray as control. Twelve fruits of uniform size were selected from each plot. Four chemicals with two concentration levels i.e. calcium chloride (1% and 2%), sodium benzoate (0.25% and 0.5%), cycocel (0.05% and 0.1%) and gibberellic acid (0.01% and 0.02%) were sprayed on the fruit as treatment along with water spray as control. Fruits were treated with the respective chemicals at 90-100 days after flowering. The fruits were harvested (30 days after spray) when the bark of the fruit appeared to be yellowish green. Harvested fruits were kept treatment-wise in the laboratory at ambient temperature (28^0C) throughout the storage period. Fresh weights of all the fruits were taken treatment-wise in each time of analysis i.e., at 3rd, 8th, 13th and 18th day after harvest. Three fruits were taken from each treatment and after peeling each fruit, pulp was removed with a sharp knife and fresh weight of the whole lot was taken. It was ground in a mixture cum grinder. Juice was sieved and weight was taken and kept in a beaker. Total sugars and titrable acidity were determined in triplicates (AOAC 1980). This continued upto the 18th day after harvest. Data were statistically analysed.

A general trend in the present study was the reduction of weight loss with post-harvest storage (Table 1). Calcium chloride (2%) and sodium benzoate (0.5%) were the most effective treatment in reducing weight loss (12.62% and 12.38%). But in gibberellic acid 0.01% treatment, the maximum weight loss was upto 30%. Both calcium and sodium have increased the fruit firmness and reduced the decay. Similar findings have been reported by Wasker et al (1994) in grape, Willium and Sams (1984) in apple and Misra et al (1978) in pineapple. Though there was a significant variation in juice content of the fruit on different treatments at different days after harvest, the overall loss of juice content was around 3 to 5% at the end of the storage period.

There were gradual increases in total sugars upto 13th day after harvest in the most of the treatments and reverse was the case with titrable acidity (Table 2). Total sugars were found to increase from 10.34 to 10.91% in calcium chloride (2%) treated fruit and decrease in acidity from 0.70 to 0.47% from the 3rd day after harvest to the 13th day. There was gradual loss of fresh weight of fruit and starch had slowly changed to sugar on storage. Organic acids were increasingly utilized as respiratory substrate, thereby decreasing acidity, Bhular et al (1987) observed similar findings in kinnow mandarin fruits. Retension of increasing sugars and loss of acidity were significantly higher in gibberellic acid (0.01%) treated fruit. Nangial et al (1997) observed similar effect in grapes. The fruits remained normal upto the 8th day after harvest and towards the 13th day rotting started in almost all the treatments except calcium chloride-treated fruits. It reduced rotting significantly compared to those of other treatments. It was found that calcium chloride at 2% concentration was the most effective treatment in arresting the rotting for extended period keeping intact the other important quality attributes like sweetness and acidity.

*Corresponding Author

TABLE 1. EFFECT OF PRE-HARVEST CHEMICAL TREATMENT ON CHANGES IN JUICE CONTENT OF PINEAPPLE FRUIT

Treatment	Fruit weight with crown, g				Total weight loss, %	Juice percentage				Total juice loss, %
	Days after harvest					Days after harvest				
	3	8	13	18		3	8	13	18	
Calcium chloride (1%)	1.08	1.01	0.98	0.91	15.75	76.95	73.92	72.49	72.63	5.22
Calcium chloride (2%)	1.03	1.06	0.90	0.90	12.62	73.86	72.98	72.01	71.03	3.83
Sodium benzoate (0.25%)	1.20	1.17	1.10	0.98	18.33	72.87	72.07	71.29	70.57	3.15
Sodium benzoate (0.50%)	1.05	1.05	0.99	0.92	12.38	72.79	71.98	71.00	70.00	3.81
Cycocel (0.05%)	0.91	0.86	0.82	0.75	17.58	73.91	73.01	72.51	71.65	3.87
Cycocel (0.10%)	0.98	0.88	0.85	0.75	23.47	73.88	72.91	72.01	71.01	3.88
Gibberellic acid (0.01%)	1.30	1.01	0.97	0.91	30.00	74.00	73.16	72.53	71.67	3.14
Gibberellic acid (0.02%)	1.09	1.03	0.99	0.90	17.43	73.37	72.99	72.01	71.03	3.83
Control	1.03	1.02	0.97	0.85	17.77	73.45	72.33	70.79	69.81	4.96
CD at 5%	0.14	0.06	0.06	0.08	-	0.02	0.04	0.02	0.06	-

TABLE 2. EFFECT OF PRE-HARVEST CHEMICAL TREATMENTS ON PHYSICO-CHEMICAL CHANGES IN PINEAPPLE FRUIT

Treatment	Total sugars, %				Titrable acidity, %				Rotting %			
	Days after harvest				Days after harvest				Days after harvest			
	3	8	13	18	3	8	13	18	3	8	13	18
Calcium chloride (1%)	10.58	10.77	11.37	11.30	0.69	0.65	0.51	0.33	Nil	Nil	Nil	73.44
Calcium chloride (2%)	10.34	10.54	10.91	10.90	0.70	0.66	0.57	0.47	Nil	Nil	Nil	73.94
Sodium benzoate (0.25%)	10.31	10.49	10.60	10.46	0.71	0.66	0.55	0.48	Nil	Nil	12.96	84.18
Sodium benzoate (0.50%)	10.24	10.40	10.48	10.38	0.69	0.65	0.60	0.48	Nil	Nil	11.31	84.99
Cycocel (0.05%)	10.67	11.11	11.65	11.78	0.69	0.60	0.54	0.40	Nil	Nil	8.41	78.19
Cycocel (0.10%)	10.49	10.62	10.99	10.98	0.70	0.65	0.58	0.44	Nil	Nil	10.10	81.35
Gibberellic acid (0.01%)	10.76	11.50	12.51	13.32	0.58	0.54	0.45	0.31	Nil	Nil	11.11	73.53
Gibberellic acid (0.02%)	10.45	10.79	11.12	11.05	0.60	0.58	0.48	0.42	Nil	Nil	10.00	74.38
Control	10.42	10.92	10.87	10.36	0.71	0.68	0.60	0.50	Nil	Nil	46.29	88.89
CD at 5%	0.05	0.04	0.03	0.03	0.02	0.02	0.02	0.02	-	-	7.40	1.97

References

Anon (1978) Annual Report, All India Co-ordinated Fruit Improvement project on Pineapple, Assam Agricultural University, Jorhat

AOAC (1980) Official Methods or Analysis. Association of Official Analytical Chemists. Washington DC

Akamine CR, Goo T (1963) Respiration and ethylene production during ontogony of fruit. J Am Soc Hort Sci 98 : 381-383

Bhangia PK. Lanka PC (974) Effect of pre- and post-harvest treatments on storage life of sapota fruits CV Oval. The Orissa Hort 22 (1 & 2) : 54-57

Bhular JS, Khoklar UV, Agnihorti RP (1987) Storage behaviour of kinow mandarin. Punjab Hort J 21(1) : 27-31

Khader SE (1991) Effect of pre-harvest application of GA_3 on post-harvest behaviour of mango fruit. Scientia Hort 47 : 317-321

Misra AK, Sailo E, Ahmed F (1978) The effect of post-harvest application of benzoic acid and 2, 4-D on extending the shelf life of pineapple. Plant Sci 10 : 187-188

Nangia RP, Bhutani VP, Sitkara S (1977) Fruit quality of parlette grape as influenced by GA_3, alone and in different combination and coconut milk. Viticulture in Tropic, HSI Bangalore, pp 245-249

Wasker DP, Damamo SV, Maralkar SD, Gaikwad RS (1974) Effect of preharvest spray of calcium on extending the shelf life of grape. The Orissa J Hort 22 (1&2) : 51-53

Willium S, Sams CE (1984) The effect of post-harvest infiltration of calcium, magnesium or strontium on decay, firmness, respiration and ethylene production in apple. J Am Sos Hort Sci 112(2) 300-305

Received 10 May 2000; revised 22 March 2001, accepted 10 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 55-58

Biochemical Characterization of Aromatic and Non-aromatic Rice Cultivars

S. BASAK, R.S. TYAGI AND K.N. SRIVASTAVA*

Division of Biochemistry, Indian Agricultural Research Institute,
New Delhi - 110 012, India.

Six rice varieties, two each of non-basmati aromatic, basmati aromatic and non-aromatic rice samples were characterized by means of solubility fractionation and gel filtration of their proteins. Protein contents of six milled rice samples ranged from 5.60% to 9.09% on fresh weight basis. Solubility fractions of the rice proteins showed albumin : globulin : prolamin : glutelin in the proportions of 9.7 : 13.5 : 3.3 : 73.4, 12.4 : 18.0 : 5.4 : 64.2 and 14.2 : 18.9 : 3.0 : 63.8 respectively for the above varietal types. The elution pattern of the total rice proteins and their fractions on Sephadex G-150 column showed that fraction I protein, eluted in the void volume was mainly glutelin having molecular weight greater than 3,00,000. On the basis of the percentage distribution of area, the fraction I constituted about 64.4% in one variety of sample, while about 48% in other two varieties. High molecular weight glutelin was found more in non-basmati aromatic varieties as compared to others.

Keywords : Rice protein, Aromatic rice, Solubility fractionation, Gel filtration, Void volume

Rice is the staple food for over 60% of the world's population. It is second only to wheat in terms of annual production for food use (Fujino 1994). Attempts have been made in different laboratories to introduce the aromatic flavour in non-basmati high yielding rice varieties because the yield of basmati rice is not satisfactory. Over 150 organic compounds are reported to exhibit aroma in rice (Buttery et al. 1983). Therefore, identification of aromatic varieties on the basis of chemical analysis is difficult. The present investigation was carried out to understand the proteins responsible for providing aromatic character in rice cultivars.

Six rice varieties, 'PB-1', 'Bas-370' (basmati aromatic), 'P-1176', 'NIRRI' (non-basmati aromatic) and 'Pusa-834', 'Pusa-677' (non-aromatic) procured from the Division of Genetics and Plant Breeding, Indian Agricultural Research Institute, New Delhi. Varieties were shelled in Satake hulling (THU-35) machine and brown rice was ground by using Tecator (200 mesh size). This powder was defatted with petroleum benzene by keeping overnight at room temperature and dried in an oven at 40^0C for 12 hrs. and stored in airtight containers at 4^0C till further use. Nitrogen content of brown rice protein was determined by Micro-kjeldahl method (AOAC 1965). From this, protein content was calculated using the factor 5.95. The Osbourne (1907) method as modified by Horiguchi and Kitagishi (1976) was used to prepare the various solubility fractions. Protein was extracted by grinding fresh grains in 0.05 mM Tris-HCl buffer (pH 8.2) in the ratio of 1 : 2.5 (w/v). The suspension was filtered through a layer of cheesecloth. The filtrate was centrifuged at 12,000 g for 15 min at room temperature. The extracted soluble protein of 'P-1176', 'PB-1' and 'Pusa-834' were loaded on a column (2 x 65 cm) of Sephadex G-150 using 100 mM Tris-HCl buffer (pH 8.2) as eluent. Each protein fraction was eluted continuously with buffer at a flow rate of 15 ml/h. The eluent was collected in fractions of 2 ml per tube. The absorbance at 280 nm was recorded in Hitachi Spectrophotometer.

**Corresponding Author*

Protein fractionation : The protein content of all the varieties found from 5.6% to 9.09% (Table 1). Higher extraction of efficiency was recorded in 'P-1176' and lower in 'Bas-370'. Though the protein contents of these varieties agreed with those of Villareal and Juliano (1978), the extraction efficiencies were found to be higher than those observed by Damardjati et al (1985). Bietz (1979) showed that extraction efficiency depended on the sequence in which the solvents are used, the vigour of extraction and the condition of the starting material. Therefore, the higher extraction efficiency obsreved in this study may be due to finer particle size of the sample and longer period of extraction. Similar to the observation of Damardjati et al (1985) the extraction efficiency did not correlate with the protein content of the milled rice.

Solubility fractionation : Soluble fraction of rice protein presented in Table 1 showed that glutelin constituted the major fraction and prolamin as the minor fraction. There was little difference in glutelin percentage of 'PB-1' and 'Pusa-834'. Glutelin percentage was considerably higher in 'P-1176'. Higher albumin percentage was recorded in 'Pusa-677' followed by 'Bas-370' and 'Pusa-834'. Globulin percentage was higher in 'Pusa-834' as compared to other varieties. Prolamin was not varied among these varieties. Many workers found glutelin as a major storage, high molecular weight (HMW) protein of rice endosperm whereas alcohol soluble prolamin remained as minor fraction (Padhye and Salunkhe 1979; Iwasaki et al. 1982; Wen and Luthe 1985). Thus the present finding agreed with the finding of the above workers.

Gel chromatography of soluble protein : The elution patterns of 'P-1176', 'PB-1' and 'Pusa-834' are shown in Fig. 1-3. Totally, six peaks were recorded in 'P-1176' among which I, IV and V were the major peaks while II, III and VI were the minor peaks (Fig 1). Elution pattern of 'PB-1' showed six peaks but I and II appeared to be same as both were eluted in void volume (Fig 2). In this case, sum of both these peaks corresponded to the peak I of other varieties. However, total number of five peaks were observed in 'Pusa-834' (Fig 3). The three corresponding prominent peaks were I, III and IV. Peak

TABLE 1. PROTEIN FRACTIONS EXTRACTED FROM MILLED RICE OF AROMATIC AND NON-AROMATIC VARIETIES

Variety	Type	Protein content[a]	Protein fraction[a]					Extraction efficiency[b]
			Albumin	Globulin	Prolamin	Glutelin	Total	
'P-1176'	NBA	6.75	0.50 (9.7)	0.69 (13.5)	0.17 (3.3)	3.76 (73.4)	5.12	75.9
'NIRRI'	NBA	5.60	0.40 (11.8)	0.52 (15.3)	0.16 (4.7)	2.32 (68.2)	3.40	60.7
'PB-1'	BA	6.97	0.57 (12.4)	0.83 (18.0)	0.25 (5.4)	2.96 (64.2)	4.61	66.1
'Bas-370'	BA	6.99	0.59 (14.6)	0.61 (15.1)	0.12 (3.0)	2.71 (67.2)	4.03	57.7
'Pusa-834'	NA	9.09	0.83 (14.2)	1.10 (18.9)	0.18 (3.0)	3.72 (63.8)	5.83	64.1
'Pusa-677'	NA	5.93	0.54 (14.8)	0.64 (17.6)	0.18 (4.9)	2.28 (62.6)	3.64	61.4

[a] Results are expressed as g protein / 100 g fresh weight
[b] Extraction efficiency is percentage extracted protein fraction from total milled rice protein
Values expressed in parenthesis indicate relative percentage of protein fraction
NBA = Non-basmati aromatic; BA = Basmati aromatic; NA = Non-aromatic

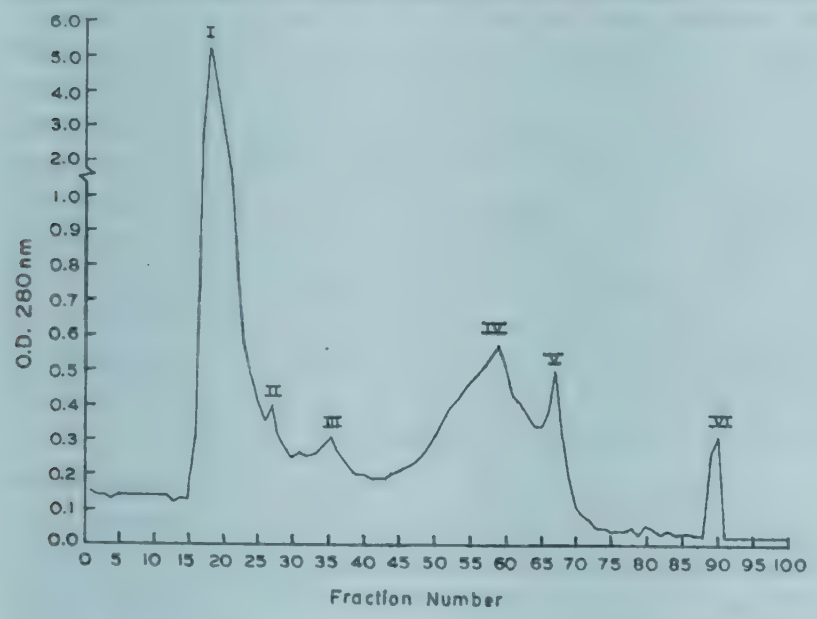

Fig. 1. Non-basmati aromatic rice protein (Variety 'P-1176')

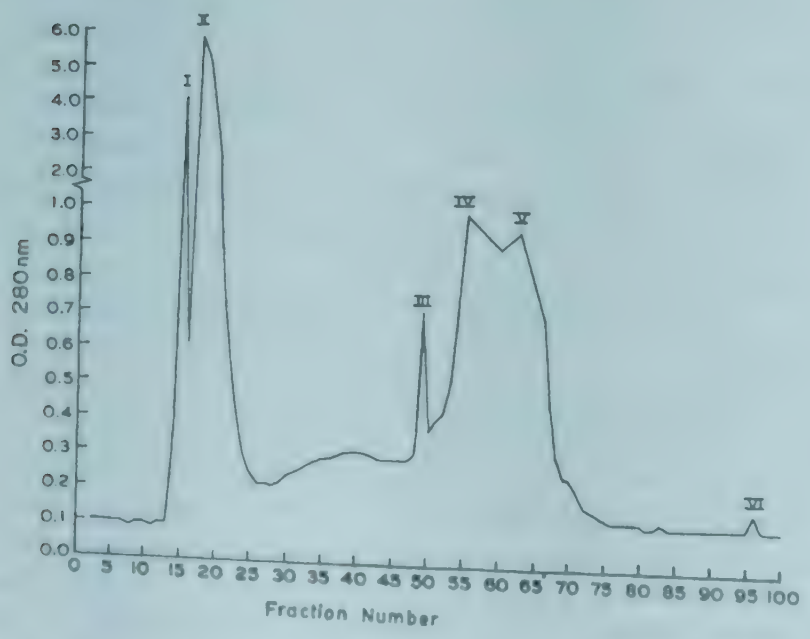

Fig. 2. Basmati aromatic rice protein (Variety 'PB-1')

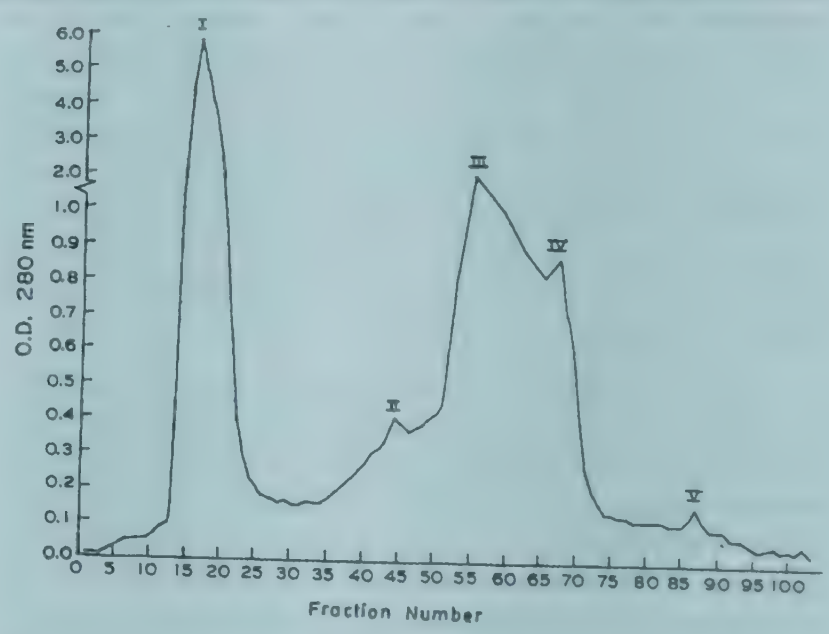

Fig. 3. Non-aromatic rice protein (Variety 'Pusa 834')

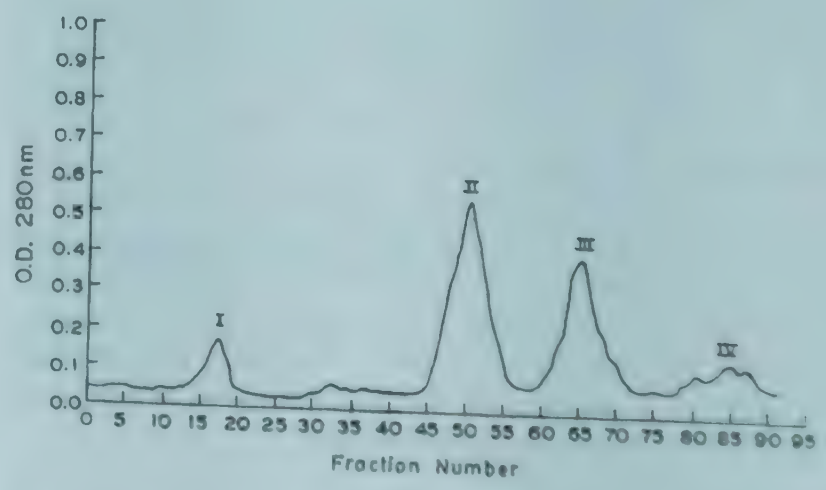

Fig. 4. Rice : Albumin + Globulin fraction (Variety 'P-1176')

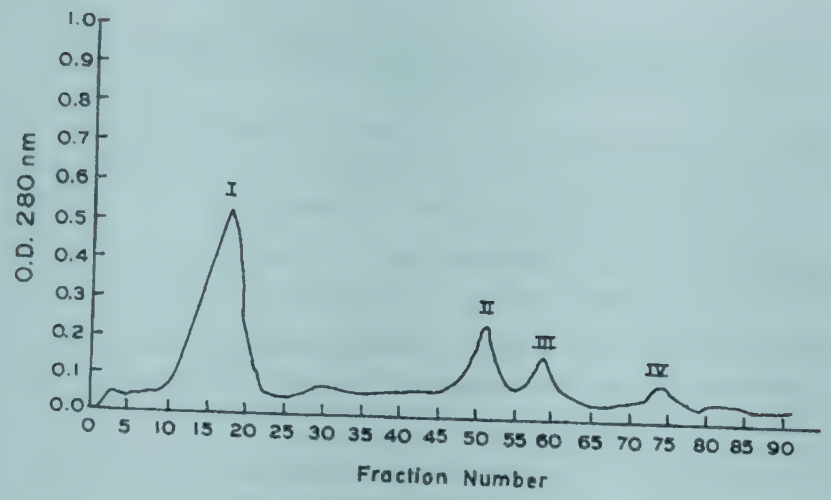

Fig. 5. Rice glutelin fraction (Variety 'P-1176')

II and V were the minor peaks. The elution pattern each of albumin + globulin and glutelin (Fig 4-5) fraction showed four peaks. In both the cases I, II and III were major peaks and peak IV was the minor one. The elution volume of all these peaks, the ratio of elution volume to total bed volume and percentage distribution of these peaks are presented in Table 2. The elution patterns of all the three varieties showed that fraction I protein, which constituted the major fraction, was eluted in the void volume of the column and therefore, has the higher molecular weight (MW) than other fractions. This fraction represented about 65% of the total eluted protein in 'P-1176' while about 48% in other two varieties. On comparing these elution profiles with those of albumin + globulin and glutelin of variety 'P-1176', it is obvious that this predominant fraction I protein was mainly glutelin and other two major peaks of these varieties corresponded to mainly albumin and globulin (Table 2). These results are in conformity with those presented in Table 1 i.e. 'P-1176' exhibited more glutelin percentage than 'PB-1' and 'Pusa-834'. The albumin and globulin fractions were found less in 'P-1176' than other two varieties. The ratio of elution volume to total bed volume (ve/vt) of the fraction I protein was about 0.16 while other two prominent fractions had the corresponding ve /vt about 0.54 and 0.66 (Table 2). Since the fraction I protein was eluted in the void volume, the protein was beyond the exclusion limit of G-150. So the molecular weight of this protein was more than 3,00,000. Damardjati et al (1985) also obtained a major peak in the void volume (ve / vo=1), when the soluble protein was fractionated on Sephadex G-100. Palmiano et al (1968) reported that the rice glutelin on Sephadex 4B exhibited a single major peak corresponding to MW 6,00,000. It is likely that fraction I protein in this study may correspond to major single peak of rice glutelin as observed by Palmiano et al (1968). It is clear from Table 2 that the variety 'P-1176' had higher relative peak area than other two varieties (which had almost same peak area). This shows that non-basmati aromatic variety has considerably more high MW protein and relatively less low MW protein. Juliano (1972) reported three rice protein fractions of salt extract albumin + globulin by gel filtration on Sephadex G-100. He obtained peaks corresponding to approximate a MW of 2,00,000. Similar values were reported by Mitsuda et al (1967) on Sephadex G-150. The elution pattern of rice albumin +

TABLE 2. ELUTION VOLUME (Ve) TO TOTAL BED VOLUME (Vt) RATIO OF DIFFERENT FRACTIONS SEPARATED ON THE BASIS OF MOLECULAR WEIGHT IN SEPHADEX G-150 (Vt = 204 ml)

Protein fraction	Elution volume Ve, ml	Ve/Vt	Percentage distribution of area
Var. 'P-1176' (Non-basmati aromatic)			
I	32	0.16	64.4
II	54	0.26	3.5
III	70	0.34	5.0
IV	112	0.55	16.3
V	136	0.66	8.0
VI	180	0.88	1.8
Var. 'PB-1' (Basmati aromatic)			
I	30	0.15	11.3
II	34	0.17	37.1
III	98	0.48	3.0
IV	110	0.54	24.2
V	132	0.65	23.2
VI	192	0.94	0.9
Var. 'Pusa-834' (Non-aromatic)			
I	32	0.16	48.0
II	88	0.43	5.6
III	110	0.54	29.6
IV	134	0.66	16.0
V	174	0.85	1.0
Var. 'P-1176' (Albumin + Globulin)			
I	36	0.17	8.8
II	104	0.51	47.9
III	130	0.64	34.8
IV	170	0.83	8.3
Var. 'P-1176' (Glutelin)			
I	36	0.18	71.3
II	106	0.52	16.5
III	118	0.58	7.8
IV	148	0.73	4.4

globulin fraction as reported in this study is in agreement with the above workers (Fig 4). The peak I (minor peak) of albumin + globulin fraction may perhaps be the glutelin contamination in this fraction. The peak II, which was the most predominant one, appears to be same of around 2,00,000 as observed by above workers. It is based on the fact that MW of this major protein fraction is less than 3,00,000 because it falls within the exclusion limit of G-150 and ve/vt ratio of this protein fraction was about same as described by Juliano (1972). Iwasaki et al (1982) fractionated the albumin and globulin fractions on Sephadex G-150 column and observed four peaks in case of albumin. As prolamin was very minor fraction and

constituted only about 4% of the total extractable protein, no further study was contemplated.

Higher glutelin content in non-basmati aromatic varieties showed the likely involvement of high molecular weight protein in providing non-basmati aromatic character. Further investigation is required to conclude on the real role of this high molecular weight protein. The albumin and globulin fraction was lower in non-basmati aromatic varieties as compared to other varieties.

References

AOAC (1965) Official Methods of Analysis, 10th edn. Association of Official Analytical Chemist, Washington DC

Bietz JA (1979) Recent advances in the isolation and characterization of cereal protein. Cereal Food World 24 : 199-206

Buttery RG, Liang LC, Juliano BO (1983) Cooked rice aroma and 2-acetyl-1-pyrroline. J Agric Food Chem 31 : 823-826

Damardjati DS, Soekarto ST, Nur A, Siwi BH (1985) Evaluation of protein quality and properties in six varieties of Indonesian rice. Indonesian J Crop Sci 1 : 1-20

Fujino Y (1994) Utilisation of rice in Japan. Ernahrungs Forschung 39 : 65-81

Horiguchi T, Kitagishi K (1976) Protein metabolism in rice seedling. Soil Sci Plant Nutr 22 : 327-333

Iwasaki T, Shibuya N, Suzuki T, Chikabu S (1982) Gel filtration and electrophoresis of soluble rice proteins extracted from long, medium and short grain varieties. Cereal Chem 59 : 192-195

Juliano BO (1972) Rice Chemistry and Technology. Houston DF ed. Am Assoc Cereal Chemists. St. Paul, Minn, p 16

Mitsuda H, Murakmi K, Kusano T, Kishida H (1967) Studies on the proteinaceous subcellular particles in rice endosperm. Agric Biol Chem 31 : 293-300

Osbourne TB (1907) The proteins of wheat kernel. Carnegie Institute, Washington Publ 84 Judd and Detweiler : Washington DC

Padhye VW, Salunkhe DK (1979) Extraction and characterization of rice proteins. Cereal Chem 56 : 389-393

Palmiano EP, Almazan AM, Juliano BO (1968) Physio-chemical properties of protein of developing and mature rice grain. Cereal Chem 45 : 1-6

Villareal RM, Juliano BO (1978) Properties of glutelin from mature and developing rice grain. Phytochem 17 : 177-182

Wen TN, Luthe DS (1985) Biochemical characterisation of rice glutelin. Plant Physiol 78 : 172-177

Received 4 December 1999; revised 23 April 2001; accepted 31 July 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 59-61

Microbiological Evaluation of Street Foods in Bhubaneswar

A. DAS MOHAPATRA, C.C RATH*, S.K. DASH AND R.K. MISHRA

Centre for Post Graduate Studies in Microbiology,
Orissa University of Agriculture and Technology,
Bhubaneswar - 751 003, India.

The investigation was carried out on the bacterial load in different street foods like *Gup Chup (Pani Puri or Gol Gopa), Chat, Dahibara,* ice cream sold in the city of Bhubaneswar. The total bacterial load, total aerobic bacterial load and presence of coliforms in different food samples were determined by employing total plate count (TPC), pour plate (PP), spread plate (SP) and most probable number (MPN) techniques, respectively. The highest bacterial load was recorded in *Gup Chup* followed by *Chat* and ice cream and *Dahibara.* The predominant bacteria isolated from the samples were species of *Bacillus, Staphylococcus, Shigella* and *E. coli.* The antibiogram pattern of these isolates is also presented.

Keywords : Bacterial load, Street foods, *Bacillus, Shigella, Staphylococcus, E. coli.*

Being ubiquitous in distribution, microorganisms can gain entry into the food chain from various sources during different stages of their processing, storage and serving. Besides, by providing a suitable nutritional and physical environment for growth and multiplication of microorganisms, the foods possess an inherent capacity to sustain them in large numbers. The possible sources of contamination of foods include environmental sources such as soil, dust and air (Heldman 1974) water (Kapdia 1984) etc. biotic sources (e.g. food handlers having illness or unhygienic practices) [Kapdia 1984; Spika 1987]; animals (Cacciapuoti 1987) other miscellaneous sources that include laundering facilities used by food handlers (Solberg et. al., 1976); raw materials and ingredients used in preparing foods (Terry and Overcast 1976); spices (Holzapfel and Baxter 1982) and also the utensils and other articles used during serving of foods. However, among all other kinds of foods consumed by human beings today, the street foods are exposed to the abundant sources of contamination. Hence, the chances of foodborne illness associated with these street foods are maximum.

In Bhubaneswar (capital city of Orissa), a large section of population belonging to all age groups (from school going children to office going and business men) have access to these street foods daily from early morning till late evening. Being the pilgrimage centre, the tourist turnout is the second highest in India (ITDC, 1999). Taking into consideration the threat posed by the killer foodborne diseases and the rapidly flourishing demands of street foods, the present work was undertaken to assess the bacteriological quality and edibility of four kinds of popular street foods [*Gup Chup* (also known as *Panipuri* or *Golgopa*), *Chat, Dahibara,* ice cream] marketed throughout the year in Bhubaneswar city. The antibiogram pattern of the isolates was also studied.

Collection of samples : A total of 105 samples consisting 30 each of *Gup Chup, Chat, Dahibara* and 15 ice cream samples were collected from vendors of different localities of Bhubaneswar. Samples from vendors were collected from the top, middle and bottom of their stocks in three pre-sterilised screw cap vials, and in the laboratory they were thoroughly mixed under aseptic conditions to get more or less homogeneous and unbiased sampling.

Bacteriological analysis : The total viable bacterial load in *Gup Chup, Chat, Dahibara* was estimated by pour and spread plate methods (Marth 1987), while that of ice cream sample was estimated by pour plate method of Fung and Chain (1991). The samples were plated on Nutrient agar (NA), MacConkey agar (MA) and Xylose Lysine Deoxycholate (XLD) agar media. The latter method is ideal for maximum recovery of heat sensitive psychrotrophs in frozen samples. The aerobic bacterial load was assessed by spread plate method (Jay 1987).

The statistical estimation of the most probable number (MPN) of coliform bacteria in the original samples were calculated on the basis of fermented tubes, employing McCardy's table (WHO 1961; Manja et al. 1982), using Brilliant Green Lactose Bile Broth (BGLB) and confirmation was done by streaking on Eosine Methylene Blue (EMB) agar plates.

The bacterial cultures were identified based on their morphological, cultural and biochemical characteristics (Collins and Lyne 1970). The antibiotic sensitivity pattern of important and predominating bacterial isolates to 10 different standard antibiotics was evaluated by the disc diffusion technique of (Bauer et al. 1966) on Mueller - Hinton agar plates.

TABLE 1. MEAN BACTERIAL POPULATION PER UNIT QUANTITY OF DIFFERENT SAMPLES

Sample	Mean total viable bacterial load cfu/ml	Mean total aerobic bacterial load cfu/ml	MPN count of coliforms MPN/100ml
Gup Chup	4.98×10^4	2.11×10^5	19.17
Ice cream	6.01×10^4	2.95×10^4	10.82
*Dahibara**	5.40×10^4	2.21×10^4	12.99
*Chat**	7.62×10^4	4.63×10^4	16.35

*Expressed as cfu/g

*Corresponding Author

TABLE 2. ANTIBIOTIC SENSITIVITY OF COMMON BACTERIAL ISOLATES

Isolate	No. of isolates tested	No. of isolates sensitive									
		Cf	Nx	Pf	C	S	Tr	T	Na	G	Do
Shigella spp.	6	5 (83.33)	5 (83.33)	-	2 (33.33)	2 (33.33)	1 (16.66)	4 (66.66)	4 (16.66)	5 (83.33)	4 (66.66)
Staphylococcus spp.	6	5 (83.33)	4 (66.66)	-	-	2 (33.33)	-	3 (50.00)	1 (16.66)	3 (50.00)	4 (66.66)
E. coli	6	6 (100.00)	6 (100.00)	3 (50.00)	2 (33.33)	-	-	3 (50.00)	2 (33.33)	4 (66.66)	4 (66.66)
Bacillus spp.	10	9 (90.00)	8 (80.00)	6 (60.00)	5 (50.00)	3 (30.00)	2 (20.00)	3 (30.00)	1 (10.00)	3 (30.00)	4 (40.00)

Values in parenthesis represent % sensitivity

Cf = Ciprofloxacin (30 mcg), Nx = Norfloxacin (10 mcg),
Pf = Pefloxacin (5 mcg), C = Chloramphenicol (30 mcg),
S = Streptomycin (25 mcg), Tr = Trimethoprim (30 mcg),
T = Tetracyclin (30 mcg), Na = Nalidixic acid (30 mcg),
G = Gentamycin (30 mcg), Do = Doxycyclin (10 mcg).

In the present study, the bacterial load and mean aerobic bacterial load per unit quantity of samples were found to be higher in *Gup Chup* samples than those in *Chat,* ice cream and *Dahibara* (Table-1). Water, the second largest source of microorganisms after soil, is the major constituent of the served *Gup Chup.* The prolonged unhealthy storage of unused *Gup Chup* water (processed water, served with *Gup Chup*) as well as its pasty ingredients by the vendor, offers wider scope of contamination. Besides, repeated churnings of *Gup Chup* water by the vendor during each time of serving not only result in repeated addition of bacteria from the hand and skin but also favour the growth and multiplication of aerobic bacteria by increasing the dissolved oxygen in the former. On the other hand, the extreme temperature involved in *Chat* (too hot) and ice cream (too cold) do not favour the multiplication of most of the bacteria. This justifies their comparatively lower bacterial load than *Gup Chup* samples, which have normal temperature.

However, addition of raw vegetables (onion, cucumber etc.), unhygienically stored *papads, namkins, chatney* and water diluted sauce during serving of *Chat* reduces its final temperature and also provides wider scope of contamination from these additives. This may account for the comparatively higher bacterial load in *Chat* in comparison to ice cream, as there is no such addition involved while serving ice cream. Besides, the lowest bacterial load in *Dahibara* may be attributed to the unfavourable acidic environment created by *Dahi* (due to presence of lactic acid) and the inhibitory activity of *Lactobacillus* species present abundantly in *Dahi,* (Mohanon et al. 1984) against a wide range of other bacteria species (Gandhi and Nambudripad, 1975; Gilliland and Speck 1989; Gupta et al. 1996). The contaminated water is usually the major source of coliform group of organisms. The coliform counts in the present investigation precisely coincide with the degree of use of water during, processing or serving of different street foods. This corroborates the use of unpotable water by the vendors, most of whom were found to be illiterate and ignorant about the basics of public health hygiene and sanitation during interaction with them.

Among 112 bacteria isolates selected from 105 processed samples, *Bacillus* spp. had their highest incidence in all 4 kinds of samples. This may be due to the ubiquitous distribution of *Bacillus* spp. in soil, water, air and other places, and their resistance to adverse environmental conditions such as heat, radiation, and desiccation owing to their spore forming nature.

The major source of *Staphylococcus* spp. are suppurated lesions of human beings (nasal and other mucous secretions and skin). In the present study, the occurrence of *Staphylococcus* spp. in different kinds of samples could be due to the extent of direct use of vendor's hands during serving of the respective street foods.

The results of antibiograms of four important and predominating bacterial isolates revealed that maximum number of isolates were sensitive to ciprofloxacin, norfloxacin, doxycycline, gentamycin and pefloxacin, and resistant to nalidixic acid and trimethoprim (Table 2). These results suggested a wide variation in the spectra of sensitivity and resistance of different bacteria. This may be due to continuous exposure of these microorganisms to various antibiotics and their adaptation to the environment by the development of mutant strains among the bacterial population.

In this investigation, the coliform count of the street food samples was exceeding the potable limit prescribed by WHO in most cases. This may be one of the important causes for the high incidences of chronic intestinal ailments reported in the working class of the city.

References

Bauer AW, Kirby WMM, Sherris JC, Turk M (1966) Antibiotic susceptibility testing by standard single disc method. Am J Clin Patol 45 : 493-496

Cacciapouti B (1987) A water borne outbreak of leptospirosis. Am J Epidemiol 129(3) : 5.5-545

Collins CH, Lyne PM (1970) Microbiological Methods. 6th edn. Butterworths London pp 66-72

Fung DYC, Chain VS (1991) Comparative analysis of redigel and aerobic plate count methods for viable cell counts of selected foods. Food Microbiol 8 : 299-301

Gandhi DN, Nambudripad VKN (1975) A note on antimirobial properties of *Dahi* (curd). Ind J Dairy Sci. 28 : 72-73

Gilliland SE, Speck ML (1975) Inhibition of psychrotrophic bacteria by *Lactpobacilli* and *Pedicococci* in non-fermentative refrigerated foods. J Food Sci 40 : 903-905

Gupta PK, Mital BK, Garg SK (1996) Inhibitory activity of *Lactobacillus acidophilus* against pathogen in milk. J Food Sci Technol 33(2) 147-148

Heldman DR (1974) Factors influencing air borne contamination of foods. J Food Sci 39 : 262-969

Holzapfel WH, Baxter R (1982) A microbiological investigation of selected species, herbs and food additives in South Africa. J Sci 47, 570-574

Indian Tourism Development Corporation (1999) Annual Report, pp 27-31

Jay JM (1987) Modern Food Microbiology, CBS Publishers, New Delhi, pp 317-329

Kapdia CR (1984) A common source of epidemic of mixed bacterial diarrhorea with secondary transmission. Am J Epidemiol 102 : 743-749

Manja KS, Maurya MS, Rao KM (1982) A simple field test for the detection of faecal pollution in drinking water. Bull World Hlth Orgn 60 : 797-801

Marth EH (1978) Standard Methods for Examination of Dairy products, 14th ed. American Public Health Association, Washington DC, pp 174-179

Mohanan KR, Shankar PA, Laxminarayana H (1984) Microflora of *Dahi* prepared under household conditions of Bangalore. J Food Sci Technol 231 : 45-46

Solberg M, Miskimin DK, Kramer R, Riha WE Jr, Franke WC, Bauchanan RL, O' Leary V, Berkowitz K (1976) Assurance of food microbiological safety in a university feeding system. J Milk Food Technol 36 : 200

Spika JS (1987) Shigellosis int a Carribbean report. Am J Epidemiol 126(6) : 173-180

Terry RC, Overcast WW (1976) A microbiological profile of commercially prepared salads. J Food Sci 41 : 211

WHO (1961) European Standards for Drinking Water. World Health Organisation, Geneva, pp 716-728

Received 27 October 2000; revised 14 May 2001; accepted 12 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 62-65

Microbial Quality of Some Turkish Honeys

NUR CEYHAN AND AYSEL UGUR*

Department of Biology, Faculty of Science and Arts,
Mugla University, Mugla/Turkey.

In this survey, total plate count, total coliform bacteria, thermophilic bacteria, yeasts and molds and osmophilic yeasts were determined by the plate count method in 54 samples of monofloral honey and 30 samples of multifloral honey collected from various sources in Mugla city in Turkey. The results have shown that total microorganisms, total coliforms, thermophilic bacteria, yeasts and molds in monofloral honey samples were more than in the multifloral honeys. Osmophilic yeast count in monofloral honey samples was almost the same as in multifloral sources of honey.

Keywords : Honey, Microbial flora, Microbial quality

Honey, the nectar and sweet deposits collected from flowers, modified and stored in the honeycomb by honey bees, is a popular sweetener (Snowdon and Cliver 1996). Honey is essentially water (average 17.2%) containing fructose (average 38.4%) and glucose (average 30.3%). Honey also contains sucrose (average 1.3%) and other carbohydrates (about 12%), minerals (average 0.169%) and proteins (169 mg/100g). The pH of honey ranges from 3.4 to 6.1 with an average of 3.9, while the water activity varies between 0.5 and 0.6 (White et al., 1962). Honey has distinctive properties that inhibit or kill most microorganisms. Hence, microorganisms of interest to the honey processing industry are those that withstand the concentrated sugar, acidity and antimicrobial character of honey (Molan 1992a,b). The microorganisms indicate sanitary or commercial quality or under certain conditions, could cause illness in humans or diseases in honey bees (Snowdon and Cliver 1996).

Primary sources of microbial contamination are pollen (Gilliam 1978; Gilliam et al. 1983), the digestive tracts of honey bees (Tysset and Durand 1968; Tysset et al. 1970 a; El-Leithy and El-Sibaei 1972; Gilliam and Prest 1987), dust, air, earth and nectar (Crane 1979; Troller 1979; Graham 1992). The secondary (after-harvest) sources of contamination for honey, are air, food, handlers, cross contamination, equipment and buildings (Tysset and Rousseau 1981; Root 1983). Secondary sources of contamination could be controlled by good manufacturing practices.

New technologies and innovative uses of honey are expanding marketing opportunities. However, new microbiological requirements pertaining to quality and safety may be associated with these opportunities. Knowledge of the moisture and temperature conditions influencing growth of microorganisms in honey has long been used to control the spoilage of honey.

Test samples : A total of 84 samples were analyzed; 54 samples were of monofloral honey and 30 samples were of multifloral honey which were obtained from various apiarists of Mugla/Turkey. The plant sources of mono- and multifloral honeys is indicated in Table 1. The honeys are usually obtained from plants of several predominant species and extracted, when the particular plant species are in bloom.

Microbial counts : These were assayed by the standard plate count method (International Commission 1986; Swanson et al 1992; Bonvehi and Jorda 1993).

Total plate count : Honey (10g) was dissolved in 90 ml of 0.1% sterile distilled water. One milliliter (0.1 ml when samples had more than 3 x 10^3 cfu/g) of the solutions were poured into each petri plate, and 10-15 ml of Plate count Agar (Oxoid) was prepared as specified by the manufacturer and added. Then, the solidified plates were incubated at 30^{0}C for 72 h.

Total coliform count : One milliliter of solutions was poured into each petri dish, and 10-15 ml Endo Agar (Difco) prepared and added. When solidified, plates were incubated at 37^{0}C for 48 h - pink, pale red, bright dark red-black colonies have been counted as coliforms.

Thermophilic bacteria count : One milliliter of solutions was poured into each petri dish and 10-15 ml Dextrose Tryptone Agar (Oxoid) was prepared and added. When solidified, plates were incubated at 55^{0}C for 48 h - yellow pin point colonies were counted as thermophilic bacteria.

Yeast and mold count : One milliliter of solutions was added onto Potato Dextrose Agar (Oxoid) petri plates by a flamed cooled glass spreader. The plates were incubated at 25^{0}C for 96 h, and typical yeast and mold colonies were counted.

Osmophilic yeast count : Similar to yeast and mold counts, one milliliter of solutions was added on to Wort Agar (Merck) petri plates by a flamed cooled glass spreader. The plates were incubated at 25^{0}C for 96 h. After incubation, typical yeast colonies in this medium were counted as osmophilic yeast.

The colonies were counted using a colony counter and then the number of each microorganism group in one gram of the honey was calculated. These values were recorded as cfu/g.

The floral sources from which the honey was obtained and the size of honey samples are given in Table 1. Table 2 shows the number of total microorganisms, total coliforms,

*Corresponding Author

TABLE 1. THE FLORAL SOURCES AND SIZE OF HONEY SAMPLES

Source	Number of samples
Pine	50
Wood purple clover	1
Citrus fruits (mixed)	1
Pine + pasture	2
Pine + chestnut	1
Pine + carob	5
Pine + carob + anise	1
Pine + thyme	2
Wood purple clover	1
Pine + flower (mixed)	20

thermophilic bacteria, yeasts and molds, osmophilic yeasts contained in per gram of the monofloral honey sample and Table 3 shows the presence of these microorganism groups in the multifloral honey samples.

It was observed that the average total plate counts were 1.6×10^4 cfu/g in monofloral honeys and 8.4×10^3 cfu/g in multifloral honeys. The average total coliform counts were 1.9×10^3 cfu/g in monofloral honeys and 8.5×10^2 cfu/g in multifloral honeys. The average thermophilic bacterial counts were 1.2×10^1 cfu/g in monofloral honeys and < 10 cfu/g in multifloral honeys. The average yeast and mold counts were 1.4×10^3 cfu/g in monofloral honeys and 1.0×10^3 cfu/g in multifloral honeys. The average osmophilic yeast counts were 1.3×10^4 cfu/g in monofloral honeys and 1.1×10^4 cfu/g in multifloral honeys. It is evident that total aerobic bacteria, total coliform, thermophilic bacteria, yeast and mold counts were more in monofloral honeys than in multifloral honeys. But osmophilic yeast counts were almost same in monofloral and in multifloral honeys.

The present results show that the total microorganism in monofloral honeys (pine honeys) ranged from 1.0×10^3 to 5.8×10^4 cfu/g and in multifloral honeys ranged from 6.5×10^2 to 3.0×10^4 cfu/g. Tysset and Rousseau (1981) examined 175 samples of commercial honey from different geographical regions of France and found the range of total plate counts to be 3 - 9.5×10^3 cfu/g. In other survey, aerobic bacteria were found in 14 samples tested by Tysset et al. (1970 b), with counts ranging from 2.5×10^1 to 1.6×10^3 cfu/g.

Yeast and mold counts in monofloral honeys ranged from 1.0×10^2 - 9.0×10^3 cfu/g, in multifloral honeys ranged from 1.0×10^2 - 8.8×10^3 cfu/g. These findings are in agreement with the observation of Tysset et al. (1970 b). In this study, a range of 0 - 2.5×10^3 cfu/g of yeasts and molds were found. Similarly, combined mold and yeast counts averaging 9.0×10^1 cfu/g (range 0 - 2.5×10^3 cfu/g) were reported in a survey of 175 samples of honey (Tysset and Rousseau 1981). The presence of yeasts and molds in honey was probably because these microorganisms can grow under acidic conditions and are not inhibited by sucrose (Snowdon and Cliver 1996). The raw material (nectar), the body of the bee, apiary soil and honey-house air and equipment are the possible sources of

TABLE 2. TOTAL PLATE, TOTAL COLIFORM, THERMOPHILIC BACTERIA, YEAST AND MOLD, OSMOPHILIC YEAST COUNTS IN MONOFLORAL HONEY SAMPLES*

Honey sample no.	Total plate cfu/g	Total coliform cfu/g	Thermo-philic bacteria cfu/g	Yeast and mold cfu/g	Osmophilic yeast cfu/g
2	3.0×10^4	1.6×10^3	-	2.1×10^3	2.5×10^4
3	1.3×10^3	4.8×10^2	-	2.6×10^2	1.7×10^3
4	1.7×10^4	2.7×10^3	-	1.2×10^3	4.5×10^4
6	2.5×10^4	1.5×10^3	-	2.2×10^3	5.0×10^4
7	2.2×10^3	8.5×10^2	-	1.1×10^2	1.8×10^3
9	2.0×10^4	6.6×10^3	-	1.7×10^3	2.6×10^4
12	1.2×10^4	1.1×10^3	1.1×10^1	6.0×10^3	2.0×10^4
15	3.0×10^4	1.9×10^3	-	2.0×10^3	2.9×10^4
21	2.5×10^4	1.5×10^3	-	6.0×10^3	1.6×10^4
22	2.5×10^4	1.5×10^3	-	5.3×10^3	4.0×10^4
23	2.0×10^4	1.8×10^3	-	4.0×10^3	4.5×10^4
24	8.9×10^3	7.8×10^2	< 10	1.4×10^2	2.0×10^3
26	4.5×10^3	1.8×10^2	< 10	1.3×10^2	3.5×10^3
27	1.6×10^4	2.2×10^3	< 10	1.6×10^2	1.5×10^4
32	3.0×10^4	1.7×10^3	< 10	1.2×10^3	1.2×10^4
33	1.1×10^4	3.3×10^3	1.2×10^1	3.5×10^3	4.2×10^4
34	1.5×10^4	7.0×10^3	1.2×10^1	8.0×10^3	1.1×10^4
36	1.9×10^4	1.6×10^3	1.5×10^1	1.2×10^3	1.4×10^4
37	2.8×10^4	4.0×10^3	-	6.0×10^2	4.0×10^3
38	1.4×10^3	2.0×10^2	-	5.0×10^2	7.0×10^3
39	2.3×10^4	2.0×10^3	-	1.3×10^2	1.4×10^3
40	3.5×10^3	3.0×10^2	< 10	1.2×10^2	2.1×10^3
42	2.0×10^4	1.1×10^3	-	2.0×10^3	2.0×10^4
43	1.5×10^3	1.0×10^2	-	3.0×10^2	4.0×10^3
44	9.9×10^3	4.0×10^2	-	5.0×10^2	8.0×10^3
46	2.1×10^3	2.1×10^2	-	1.0×10^2	1.4×10^3
48	2.6×10^3	6.0×10^2	-	1.0×10^2	1.9×10^3
51	8.0×10^3	8.0×10^2	-	5.0×10^2	1.2×10^3
54	5.5×10^3	2.3×10^2	< 10	1.4×10^2	1.8×10^3
55	2.0×10^3	2.0×10^2	-	2.6×10^2	2.5×10^3
56	5.8×10^4	4.0×10^3	-	4.0×10^2	6.0×10^3
57	1.1×10^4	5.6×10^4	-	1.5×10^3	3.0×10^4
58	2.2×10^4	1.6×10^3	-	1.7×10^3	4.3×10^4
59	2.2×10^4	1.8×10^3	-	1.9×10^3	2.0×10^4
60	1.8×10^4	1.4×10^3	-	1.2×10^3	1.6×10^4
61	1.6×10^4	1.0×10^3	-	1.1×10^3	4.0×10^4
62	3.0×10^4	8.0×10^3	-	1.6×10^3	1.5×10^4
63	1.3×10^4	1.2×10^3	-	7.0×10^3	1.0×10^4
64	8.0×10^3	5.0×10^2	-	3.0×10^2	2.0×10^3
65	1.9×10^4	1.3×10^3	-	6.0×10^3	1.2×10^4
66	2.1×10^4	4.5×10^3	-	9.0×10^3	2.1×10^4
69	4.5×10^3	7.0×10^2	-	2.1×10^2	2.5×10^3
70	2.0×10^3	3.0×10^2	-	2.0×10^2	5.0×10^3
71	1.2×10^3	2.0×10^2	-	6.0×10^2	5.1×10^3
72	1.0×10^3	2.0×10^2	-	2.0×10^2	1.2×10^3
73	2.2×10^3	4.0×10^2	-	1.0×10^2	2.0×10^3
74	2.4×10^3	4.0×10^2	-	1.0×10^2	1.0×10^3
75	5.0×10^4	5.0×10^3	-	3.0×10^2	2.0×10^3
76	4.5×10^4	7.0×10^3	-	4.0×10^2	2.4×10^3
77	3.5×10^4	8.0×10^3	-	2.0×10^2	3.0×10^3
78	3.8×10^4	1.0×10^3	< 10	4.0×10^2	2.5×10^3
80	3.1×10^4	1.3×10^3	-	1.1×10^2	2.7×10^3
82	1.6×10^3	3.0×10^2	-	2.0×10^2	3.0×10^3
83	1.4×10^3	2.0×10^2	-	1.0×10^2	4.0×10^3
Lowest	1.0×10^3	1.0×10^2	< 10	1.0×10^2	1.0×10^3
Highest	5.8×10^4	8.0×10^3	1.5×10^1	9.0×10^3	5.0×10^4
Mean	1.6×10^4	1.9×10^3	1.2×10^1	1.4×10^3	1.3×10^4

* Number of microorganisms in per gram of honey samples
- No growth
< 10 Lower than 1×10

TABLE 3. TOTAL PLATE, TOTAL COLIFORM, THERMOPHILIC BACTERIA, YEAST AND MOLD, OSMOPHILIC YEAST COUNTS IN MULTIFLORAL HONEY SAMPLES*

Honey sample no.	Total plate, cfu/g	Total coliform, cfu/g	Thermo-philic bacteria, cfu/g	Yeast and mold, cfu/g	Osmophilic yeast, cfu/g
1	2.9×10^4	1.2×10^3	-	1.3×10^3	4.0×10^4
5	3.2×10^3	2.8×10^2	-	1.2×10^2	1.0×10^3
8	2.9×10^4	1.8×10^3	-	2.0×10^3	2.1×10^4
10	4.0×10^2	1.2×10^1	-	7.0×10^2	8.4×10^3
11	4.0×10^3	1.3×10^2	-	1.5×10^2	5.0×10^3
13	7.5×10^3	5.0×10^2	-	5.2×10^2	8.0×10^3
14	3.0×10^4	2.7×10^3	-	1.0×10^3	2.4×10^4
16	1.6×10^3	8.2×10^2	-	5.1×10^2	6.5×10^3
17	1.0×10^4	1.4×10^3	-	5.0×10^3	6.2×10^4
18	1.6×10^3	1.0×10^3	-	4.8×10^3	3.3×10^4
19	2.8×10^4	1.2×10^3	-	1.1×10^3	4.5×10^4
20	2.7×10^4	7.5×10^3	-	8.8×10^3	1.2×10^4
25	2.2×10^3	8.0×10^2	-	1.2×10^2	2.4×10^3
28	1.6×10^3	7.5×10^2	-	1.8×10^2	1.0×10^3
29	3.6×10^3	1.5×10^2	-	1.0×10^2	1.5×10^3
30	7.2×10^3	2.0×10^2	< 10	1.4×10^2	1.5×10^3
31	2.8×10^3	2.5×10^2	< 10	1.3×10^2	1.5×10^3
35	3.0×10^4	2.1×10^3	-	1.4×10^3	1.5×10^4
41	2.5×10^3	3.0×10^2	-	1.0×10^2	3.0×10^3
45	9.0×10^2	4.0×10^1	-	4.3×10^2	5.5×10^3
47	5.1×10^3	4.1×10^2	-	3.0×10^2	6.0×10^3
49	7.5×10^2	4.0×10^1	-	1.0×10^2	1.5×10^3
50	3.0×10^3	1.3×10^2	-	2.0×10^2	3.3×10^3
52	6.8×10^2	8.8×10^1	-	1.0×10^2	1.1×10^3
53	7.3×10^3	2.3×10^2	-	1.0×10^2	1.3×10^3
67	4.0×10^3	3.1×10^2	-	2.0×10^2	2.0×10^3
68	4.4×10^3	5.0×10^2	-	8.0×10^2	3.6×10^3
79	6.5×10^2	1.5×10^1	-	1.5×10^2	1.0×10^3
81	3.0×10^3	3.1×10^2	-	1.0×10^2	1.0×10^3
84	1.2×10^3	2.0×10^2	-	1.0×10^2	4.3×10^3
Lowest	6.5×10^2	1.2×10^1	< 10	1.0×10^2	1.0×10^3
Highest	3.0×10^4	7.5×10^3	< 10	8.8×10^3	6.2×10^4
Mean	8.4×10^3	8.5×10^2	< 10	1.0×10^3	6.2×10^4

* The Number of microorganisms in per gram of honey samples
- No growth
< 10 Lower than 1 x 10

yeasts and molds (Gilliam 1978; Crane 1979; Gilliam et al. 1983; Gilliam and Prest 1987).

Osmophilic or sugar tolerant yeasts can grow even at the limited level of water available in ripe honey and fermentation as well as granulation are the major problems in the honey industry. The osmophilic yeast counts ranging from 1.0×10^3 to 5.0×10^4 cfu/g in monofloral honeys, 1.0×10^3 to 2×10^4 cfu/g and in multifloral honeys are in agreement with the survey. Piana et al. (1991) assayed 50 samples of Italian honey and found osmophilic yeasts in the range of $1 - 3.5 \times 10^3$ cfu/g; only 34 of the samples had osmophilic yeasts. Osmophilic yeast counts ranging from $0 - 1.05 \times 10^4$ cfu/g were reported in a study of 175 samples of honey (Tysset and Rousseau 1981). Assays of 320 samples of Canadian honey demonstrated sugar-tolerant yeasts ranging from $0 - 1.0 \times 10^6$ cfu/g (Root 1983).

The present findings indicate that total plate and total coliforms in honey samples obtained from thyme + pine, pine + carob, pine + carob + anise and pine + chestnut were generally lower than honey from other florals. Yeast and mold counts and osmophilic yeast counts were almost same in multifloral and monofloral honeys.

There are intrinsic properties of honey that effect microbial growth. These include pH, moisture content, oxidation-reduction potential, nutrient content, and antimicrobial constituents. Due to the intrinsic properties of honey and control measures followed in the honey industry, honey is a product with minimal types and very low levels of microorganisms. Therefore, good manufacturing practices for beekeeping and for packing have been suggested by several workers (Crane 1979; Graham 1992). It is important to control the environmental factors and the materials carried in by the bees (Troller 1979) and to keep bees healthy (Gilliam et al. 1983; Root 1983). It is necessary to avoid inoculation of honey with undesirable microorganisms and also to avoid handling or storing the honey in such a way as to encourage microbial growth (Snowdon and Cliver 1996).

In conclusion, yeasts and molds are typically found in honey, often in low numbers. Certain bacteria will survive in honey but growth is unlikely. Vegetative forms of pathogenic bacteria were almost nil in honey.

References

Bonvehi JS, Jorda RE (1993) The microbiological quality of honey is determined by aerobic colony counts. J Food Protect 56 : 336-337

Crane E (1979) A Comprehensive Survey, Heinemann, London p 67

El-Leithy MA, El-Sibaei KB (1972) Role of microorganisms isolated from honey-bees (*Apis mellifera*) in ripening and fermentation of honey. Egypt J Microbiol 7 : 89-95

Gilliam M (1979) Bacteria belonging to the genus *Bacillus* isolated from selected organs of queen honey bees, *Apis mellifera*. J Invert Pathol 31 : 389-391

Gilliam M Prest DB (1987) Microbiology of faeces of the larval honey bee, *Apis mellifera*, J. Invert. Pathol 49 : 70-75

Gilliam M, Moffett JO, Kauffeld NM (1983) Examinations of floral nectar of citrus, cotton and Arizona desert plants for microbes. Apidologie 14 : 299-302

Graham JM (ed.) (1992) The Hive and the Honey Bee, Dadant and Sons, Hamilton. Illinois p 120

ICMSF (1986) Meaningful microbiological criteria for foods. In : Microorganisms in foods. 2 - Sampling for Microbiological Analysis : Principles and Specific Applications. International Commission on Microbiological Specification for Food. University of Toronto Press, Buffalo pp 3-15

Molan PC (1992 a) The antibacterial activity of honey 1. The nature of the antibacterial activity. Bee World 73 : 5-28

Molan PC (1992 b) The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. Bee World 73 : 59-76

Piana ML, Poda G, Cesaroni D, Cuetti L, Bucci MA, Gotti P (1991) Research on microbial characteristics of honey samples of Udine province. Riv Soc Ital Sci Aliment 20 : 293-301

Root AI (ed.) (1983) The ABC and the XYZ of Bee Culture. The A I Root Co., Medina, OH 155

Snowdon JA, Cliver DO (1996) Microorganisms in honey. International J Food Microbiol 31 : 1-26

Swanson KMJ, Busta FF, Peterson EH, Johnson MG (1992) Colony counts methods, In : Compendium of Methods for the Microbiological Examination of Foods. Vanderzant C, Splittstoesser DF (eds.), American Public Health Association, Washington, DC pp 75-95.

Troller JA (1979) Food spoilage by microorganisms tolerating low-a_w environments. Food Technol 33 : 72-75

Tysset C, Durand C (1968) Contribution to the study of the intestinal microbism of healthy foraging bees : inventory of the gram-negative bacterial populations, Fourth Report. Bull Apicole 11 : 107-118

Tysset C, Rousseau M (1981) Problem of microbes and hygiene of commercial honey. Rev Med Vet 132 : 591-600

Tysset C, Brisou J, Durand C, Malaussene J (1970 a) Contribution to the study of intestinal microbial infection of healthy honey *(Apis mellifera)* bees : inventory of bacterial populations by negative gram. Ass Diplom Microbiol Fac Pharm Univ Nancy Bull 116 : 41-53

Tysset C, Durand C, Taliergio YP (1970 b) Contribution to the study of the microbial contamination and the hygiene of commercial honey. Rec Med Vet 146 : 1471-1492

White JW Riethof ML Subers MH Kushnir I (1962) Composition of American honeys. Tech. Bull 1261, Agricultural Research Service, US Department of Agriculture, Washington, DC p 200

Received 8 November 2000; revised 14 May 2001; accepted 12 December 2001

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 66-68

Packaging and Storage Studies on Biscuits Containing Finger Millet (Ragi) Flour

A. SELVARAJ[1*], N.BALASUBRAHMANYAM[2] AND P. HARIDAS RAO[1]

[1] Flour Milling, Baking and Confectionery Technology Department,
[2] Food Packaging Technology Department,
Central Food Technological Research Institute, Mysore - 570 013, India.

Sorption studies on biscuits containing finger millet flour indicated that a moisture content of 5.0% equilibrating to 32% RH was critical with respect to storage stability of the product. Shelf life periods of biscuits were 75 and 50 days at 90% RH, 38^{0}C, when packed in double pack of polypropylene/ pearlised BOPP and metallised polyester/ poly laminate packs, respectively and over 120 days at 65% RH, 27^{0}C in both types of packs. Their sorption characteristics and shelf life were comparable to that of conventional glucose biscuits.

Keywords : Finger millet flour, Composite flour, Biscuit, Shelf-life, Packaging materials, Accelerated and ambient conditions, Moisture content, Extractable fat acidity, Sensory qualities.

Use of composite flour based on wheat, other cereals and minor millets in bakery products is becoming popular because of several economic and nutritional advantages. However, scientific information on the use of finger millet (*Eleusine coracana,* i.e. ragi*)* in bakery product is not available. Finger millet, extensively grown in Southern part of India, is widely consumed in the form of dumpling by a vast section of population. The composition of finger millet is, proteins 7.3%, fat 1.3% carbohydrate 72%, crude fibre 3.6%, and minerals 2.7% with calcium 344 mg% (Gopalan et al. 1971). It is one of the richest sources of calcium, which is about 10-fold higher as compared to other foodgrains. In view of its advantages like low cost and better nutritional quality, this institute has developed formulation of composite flour biscuit based on wheat flour and finger millet flour (Unpublished data). The present work was aimed to study the moisture sorption characteristics and keeping quality of such biscuits stored in flexible packs and the results are presented in this paper.

Formulation : Refined wheat flour (*maida*) was obtained from a pilot roller flour mill of 20 tonnes capacity of this Institute. Commercially available sugar powder (60-80 mesh), hydrogenated vegetable shortening (*marvo*), salt, lecithin and baking chemicals were procured from the local market. Finger millet flour of 90% extraction rate was obtained by milling the millet in a plate mill (*chakki*) and sieving it through 40 mesh (525 micron) sieve to retain 10% overtailings. Biscuits containing finger millet flour were prepared at pilot scale level according to the following formulation (g) : *maida*-finger millet flour blend (80:20):100, sugar powder :35, hydrogenated vegetable shortening :20, glucose powder :1, salt :1, sodium bicarbonate :0.5, acid calcium phosphate :0.75, ammonium bicarbonate :0.5, lecithin :0.5, vanilla :0.5 and water 20 ml. Conventional glucose biscuits without finger millet flour were also prepared and used as control for the studies.

Packaging : Considering the physico-chemical attributes and functionality of the packaging materials as well as the product sensitivity to the vagaries of climatic conditions in different regions, the following packaging materials were used. The water vapour transmission rates (WVTR) of the packaging materials determined according to the standard method (IS 1060:1960) are given in brackets and are expressed as g/ sq.m/ day under 90% RH gradient at 38^{0}C.

1. Double pack of (a) inside 160 gauge (40 micron) polypropyléne - PP (6.1) and (b) outside 160 gauge (40 micron) pearlised biaxially oriented polypropylene - BOPP (4.0).

2. 12 micron metallised polyester/ 15 gauge (37.5 micron) polyethylene laminate - MetPET/ Poly (2.0).

Chemical and sensory analysis : Moisture content and extractable fat acidity of biscuit samples were determined according to AACC (1983) procedures. The sensory evaluation of the biscuits for texture, taste and flavour, and overall quality was carried out by a panel of 9 trained judges who were familiar with the product using standard composite scoring test having a total score of 30 with the following individual score : texture - 10 ; taste and flavour - 10 ; overall quality - 10. According to the total scores, the products were graded as follows : very good 24 - 30 ; good 18 - 23 ; fair 14 - 17 ; poor 9 - 13 and very poor less than 9. The data were analysed according to Duncan's multiple range test (Duncan 1957).

Sorption studies : Humidity-moisture relationship studies of biscuits containing finger millet flour as well as control biscuits were carried out by exposing weighed quantities of samples in petri dishes to relative humidities (RH) ranging from 11% to 86% built inside the desiccators by using appropriate saturated salt solution (Rockland 1960). The samples were periodically weighed till they attained practically constant weight or showed signs of mould growth. After equilibrium, the samples were assessed for changes in crispness, odour and visible microbial spoilage. The equilibrium moisture content (EMC) of the product at different RHs was calculated by subtracting or adding the percentage loss or gain of moisture at different RH conditions to the initial moisture content.

Packaging and storage studies : Biscuits (100g) were filled in 105 x 165 mm size pouches, heat sealed and exposed

*Corresponding Author

TABLE 1. HUMIDITY-MOISTURE RELATIONSHIP OF BISCUITS AT 27°C

Relative humidity, % RH	Biscuit containing finger millet flour: Equilibrium moisture content, % EMC %	Remarks	Conventional glucose biscuit: Equilibrium moisture content, % EMC %	Remarks
11	2.2	Crisp; good	2.2	Crisp; good
22	3.3	Crisp; good	3.4	Crisp; good
32	5.0	Crisp; good	5.0	Crisp; good
44	6.5	Slightly soft	6.0	Slightly soft
57	8.0	Very soft	8.5	Very soft
64	9.3	Very soft	9.5	Very soft
75	14.8	Mould growth in 15 days	15.0	Mould growth in 15 days
86	19.4	Mould growth in 15 days	19.3	Mould growth in 15 days
% Initial moisture content :	1.2		2.1	
% Equilibrium relative humidity (ERH) :	7		11	
% Critical moisture content (CM) :	5.0		5.0	
% Critical relative humidity (CRH) :	32		32	

TABLE 2 : CHANGES IN CHEMICAL AND SENSORY QUALITIES OF BISCUITS DURING STORAGE

Packaging material	Storage period, days	Biscuit containing finger millet: Moisture content, %	Acidity, %	Overall quality	Conventional glucose biscuit: Moisture content, %	Acidity, %	Overall quality
Storage condition	0	1.2	0.61	V. good	1.8	0.70	V. good
92% RH, 38°C	30	3.0	0.66	Good	3.3	0.74	Good
Double pack of polypropylene & pearlised	60	4.4	0.73	Good	4.7	0.79	Good
BOPP	90	5.4	0.81	Poor	5.7	0.87	Poor
Metallised polyester / polyethylene	30	3.5	0.68	Good	3.7	0.76	Good
	60	5.7	0.77	Fair	6.0	0.80	Fair
	90	7.0	0.87	Poor	8.5	0.93	Poor
65% RH, 27°C	60	2.2	0.64	Good	2.8	0.73	Good
Double pack of polypropylene & pearlised	90	2.9	0.68	Good	3.4	0.77	Good
BOPP	120	3.3	0.71	Fair	3.9	0.81	Fair
	150	3.8	0.72	Fair	4.4	0.83	Fair
Metallised polyester / polyethylene	60	2.3	0.63	Good	2.8	0.73	Good
	90	3.0	0.66	Good	3.6	0.76	Good
	120	3.7	0.69	Fair	4.2	0.80	Fair
	150	4.2	0.71	Fair	4.6	0.83	Fair

to (i) accelerated storage conditions of 90 ± 2% RH, 38 ± 1°C (ii) Indian standard conditions of 65 ± 2% RH, 27 ± 1°C. Storage studies were carried out with both conventional and finger millet flour incorporated biscuits. Packets stored in both conditions were withdrawn at regular intervals and biscuits were analysed for moisture content, extractable fat acidity and sensory quality.

Sorption studies : Table 1 gives the data on the humidity-moisture relationship and sorption characteristics of the two types of biscuits at 27°C. With the initial moisture contents of 1.2% and 2.1%, biscuits containing finger millet flour and control ones were in equilibrium with 7% and 11% RH, respectively. As can be seen from the above data, both the products had similar sorption characteristics as well as the onset of rapid physico-chemical changes above 44% RH. Both samples equilibrated to 44%, were slightly soft with equilibrium moisture content of 6.0 to 6.5%. On the other hand, both the products equilibrated to 32% RH and having EMC of 5.0% were crisp and good. Similarly, samples equilibrated to 22% and 11% RH were very crisp and good. However, samples

equilibrated to 57% RH and 64% RH were very soft and not acceptable. At higher RH of 75% and 86%, both the products developed mould growth.

From the sorption data obtained, it can be inferred that a moisture content of 5.0% (RH 32%) is critical with respect to loss of crispness in both the products. Further, biscuits containing finger millet flour and soft dough biscuits have similar sorption characteristics and are equal to moisture content of 5% of ISI (1992) specifications for biscuits.

Storage studies - i) *Accelerated conditions of 90% RH, 38^{0}C* : Changes in moisture content, extractable fat acidity and overall quality characteristics in both types of biscuits under two different conditions of storage are presented in Table 2. Results indicated that biscuits containing finger millet flour stored in double PP pouches and Met PET/poly laminate packs under accelerated conditions of 90% RH, 38^{0}C reached to critical moisture content of 5.0% in about 75 days and 50 days respectively. Similar trend was also noticed in control soft dough biscuits. The initial extractable fat acidity of biscuits containing finger millet flour was 0.61% and it was slightly lower than that of control biscuits with 0.7%. The extractable fat acidity of biscuit containing finger millet flour increased gradually from 0.61% to 0.81 and 0.87% and that of control biscuit gradually increased from 0.70 to 0.87% and 0.93% respectively during storage in both types of packs. The increase in fat acidity in two types of biscuits in both packs over the storage period was similar and these values were lower than the maximum acidity of 1.2% specified by ISI (1992) for biscuits. Overall quality of biscuits followed a pattern similar to changes in moisture, indicating that the loss of crispness was proportional to increase in moisture. Results suggest a storage life of about 75 days and 50 days in double pack of PP/pearlised BOPP and MetPET/poly packs, respectively for both types of biscuits.

ii) Ambient storage condition of 65% RH, 27^0 C : As can be seen from Table 2, the critical moisture content of 5.0% was not reached even after 150 days in two types of packs of biscuits. The extractable fat acidities of biscuits containing finger millet flour increased from 0.61 to 0.71 and 0.72 and that of control biscuit increased from 0.70 to 0.83 in both types of packs under the ambient conditions of storage. These increases in extractable fat acidity of both types of biscuits packed in two types of packs were slow, gradual and similar over the period of storage and were well within the maximum permissible limit of 1.2%. The overall quality characteristics of biscuits containing finger millet flour was rated good upto 90 days and fair upto 150 days of storage in both types of packs. Similar trend was also observed in case of conventional glucose biscuits. However, a slight stored stale odour was noticed in both types of biscuits at the end of 150 days.

It may be concluded that the biscuits containing finger millet flour have 75 and 50 days shelf life periods in double pack of polypropylene/ pearlised BOPP and MetPET/poly respectively at 90% RH, 38^{0}C, and over 120 days at 65% RH, 27^{0}C in both types of packs. Also, the storage behaviour of biscuit containing finger millet flour could be similar to that of conventional glucose biscuits.

The authors are grateful to the Director, CFTRI, Mysore, for keen interest and encouragement in this work.

References

AACC (1983) Approved Methods, 8th edn. Association of American Association of Cereal Chemists, St. Paul, Minnesota

Duncan DB (1957) Multiple range test for correlated and heteroscedastic means. Biometrics 13 : 164-176

Gopalan C, Rama Sastri BV, Balasubramanian SC (1971) In. Nutritive values of Indian Foods : National Institute of Nutrition, ICMR Hyderabad

ISI (1966) Methods of sampling and test for papers and allied products, Part I. Bureau of Indian Standards, Manak Bhavan, New Delhi. IS : 1060-1966

ISI (1992) Biscuit - specification. Bureau of Indian Standards, Manak Bhavan, New Delhi. IS : 1011-1992

Rockland LB (1960) Standard salt solution for static control of relative humidities between 5^{0}C and 40^{0}C. Anal Chem 32 : 1375-1376

Received 3 November 2000; revised 17 May 2001; accepted 12 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 69-71

Effect of Processing and Cooking on Proximate Composition of Chickpea (*Cicer arietinum*) Varieties

M. SOOD, S.R. MALHOTRA* AND B.C. SOOD

Department of Food Science and Nutrition,
College of Home Science,
C.S.K.K.V, Palampur - 176 062, India.

Two cultivars of chickpea namely 'HPG-17' and 'C-235' varieties were evaluated for their proximate composition after subjecting them to different food processing conditions. After pressure cooking, solar cooking and parching, almost all the constituents were found to decrease in both the varieties except for fibre and carbohydrates. In general, 'HPG-17' variety was found to be better in all respects when compared with 'C-235' variety.

Keywords : Chickpea, Pressure cooking, Solar cooking, Germination, Parching, Proximate composition.

Legumes are widely grown throughout the world and their dietary and economic importance is well established. Legumes not only add variety to human diet, but also serve as an economical source of supplementary proteins for a large human population in developing countries like India where majority of the population is vegetarian (Bishnoi 1991). Chickpea (*Cicer arietinum*) commonly known as *Bengal*gram or *Garbanzo* bean is an unique legume, which is grown mainly in the Indian sub-continent, the Mediterranean basin, East Africa, Mexico and Australia. Chickpea is the world's third most important grain legume after beans and peas (FAO 1993). Chickpea is a good source of carbohydrates, proteins, minerals and trace elements and its protein quality is similar to or better than other legumes such as pigeonpea, blackgram and greengram (Williams and Singh 1987).

In India, the most common domestic methods for processing of legumes include, soaking for different periods, dehulling, ordinary cooking and pressure cooking, germination and parching. These processing methods have been reported to be beneficial for enhancing the nutritive value of various food legumes, as they reduce the contents of antinutritional factors and improve the digestibility of carbohydrates and proteins (Kataria et al. 1989). Keeping in view the effects of various cooking methods on the nutritive value of pulses, the present study was undertaken to determine the effects of cooking methods on two varieties of chickpea.

Procurement of materials : The seeds of two cultivars ('HPG-17' and 'C-235') of chickpea (*Cicer arietinum*) were procured in a single lot from the Department of Plant Breeding and Genetics, College of Agriculture, CSKKV, Palampur. The treated as well as untreated seeds were evaluated for their physico-chemical characteristics like 100 seed weight, hydration capacity and density as described below :

100-seed weight : One hundred seeds from each variety were taken at random and weighed in duplicates separately. The average weight of 100 seeds was recorded in gms.

Density : Chickpea seeds (100 g) were weighed accurately and transferred to a measuring cylinder. Then, 100 ml distilled water was added to it. Seed volume was recorded as total volume (ml) - 100 ml. Density was recorded as g per ml.

Hydration capacity : Seeds weighing 100 g were counted and transferred to a measuring cylinder of 500 ml capacity and 100 ml water was added. The cylinders were covered with aluminium foils and left overnight at room temperature. Next day, seeds were drained, superfluous water was removed with filter paper and swollen seeds were reweighed. Hydration capacity per seed was determined using the following formula :

$$\text{Hydration capacity} = \frac{\text{Weight of soaked seeds} - \text{Weight of seeds before soaking}}{\text{Number of seeds}}$$

Hydration Index : Hydration index was calculated as below :

$$\text{Hydration index} = \frac{\text{Hydration capacity per seed}}{\text{Weight of one seed (g)}}$$

Swelling capacity : Seeds weighing 100 g were counted, their volume noted and soaked in 350 ml water overnight. The volume of the seeds before and after soaking was noted with the help of a graduated cylinder. Swelling capacity per seed was determined using the following formula :

$$\text{Swelling capacity (ml/seed)} = \frac{\text{Volume after soaking} - \text{Volume before soaking}}{\text{Number of seeds}}$$

Cooking time : Seeds (100 g) of chickpea were taken in beakers fitted with condensers to avoid evaporation during boiling. Water was added in a ratio of 1:4 (w/v). Samples were stirred at 2 min interval. After 45 min, one seed was withdrawn without interrupting the boiling. Degree of cooking was tested by pressing seeds between forefinger and thumb. If seeds were felt uncooked, one seed was again tested after 5 min. This procedure continued until five seeds tested were found cooked. At this time, total cooking time was recorded.

Processing and cooking of chickpea : Grains of the two varieties of chickpea were subjected to various processing

**Corresponding Author*

methods including soaking, germination, pressure cooking, indirect cooking, solar cooking and parching. The seeds were cleaned off dust and dirt. Raw seeds were ground in an electric grinder and packed in airtight plastic containers for further analysis. Seeds were soaked in water overnight at room temperature using seed to water ratio of 1:5 (w/v). The soaked seeds were washed with water (except for parching, the samples were soaked before giving any other treatment). The soaked samples were allowed to germinate in an incubator. The samples were then dried and ground. Thereafter, the samples were stored in containers to equilibrate the moisture for further use in the investigation. After germination, the samples were indirectly cooked (steaming) in pressure cooker till done (15 min). Thereafter the samples were tray-dried and ground to pass through 80-mesh sieve and stored in airtight containers. The seed to water ratio for pressure cooking was 1:3 (w/v). The soaked chickpea seeds were cooked till done (15 min). Soaked samples of the two varieties were cooked using seed to water ratio of 1:2 for soaked samples. After an hour, one seed was withdrawn without interrupting the cooking. Degree of cooking was tested by pressing seeds between the fingers. If the seeds were found uncooked, the cooking was continued for another 30 min until 5 seeds tested were found cooked. Subsequently, the procedure was continued, the processed samples were dried in tray drier, ground and stored in airtight container for further analysis. Initially, the grains were sprinkled with a little water, with or without common salt. The chickpeas were then mixed with four times its volume of pre-heated sand (220 ± 5^0C) contained in a pan and kept on an open fire, the sand reaching a suitable temperature. The chickpea was subsequently parched by rapid mixing in the pan using a ladle. The roasted material was separated from the sand by sieving. The sample was then ground to pass through a 80 mesh sieve and stored in airtight containers for further investigation.

Proximate analysis : The proximate composition of the processed samples was then estimated by employing the standard methods of analysis (AOAC 1990). The carbohydrate content was calculated by difference. The results obtained were statistically analyzed (Panse and Sukhatme 1961).

Physico-chemical characteristics : The data related to the physico-chemical characteristics of treated chickpea varieties are given in Table 1. The weights of two varieties of chickpea were 22.80 and 11.31 g, respectively. The seed weights of the untreated as well as seeds of chickpea varied significantly. The highest seed weights (51.07 and 25.07 g) were recorded in pressure cooked chickpea seeds of both the varieties. Density values also varied significantly. A non-significant difference was observed in treated seeds of chickpea using different methods of cooking in both the varieties. The swelling capacity of 'HPG-17' was 0.69 ml per seed and 0.53 ml per seed in 'C-235' variety. The hydration capacity and hydration index were also higher in 'HPG-17' with the mean values of 0.48 and 1.64 g per seed when compared with 'C-235' having the values as 0.43 and 1.37 g per seed, respectively.

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF CHICKPEA VARIETIES

Parameters	'HPG-17'	'C-235'	CD ($P \leq 0.05$)	S.E.
100-seed weight, g	22.80	11.31	3.93	1.17
Density, g/ml	2.42	1.70	0.05	0.02
Swelling capacity, ml/seed	0.69	0.53	0.06	0.08
Hydration capacity, g/seed	0.48	0.43	0.03	9.19
Hydration index, g/seed	1.64	1.37	0.02	7.77
Cooking time, min.	46.50	62.50	2.90	0.86

TABLE 2. EFFECT OF PROCESSING AND COOKING ON PROXIMATE COMPOSITION OF CHICKPEA VARIETIES (VALUES ARE g/100g ON DRY BASIS)

Varieties	Treatments	Protein	Fat	Ash	Crude fibre	Carbo-hydrates
'HPG-17'	Raw	31.50	8.82	4.44	3.24	52.00
	Soaked	32.41	8.88	3.96	3.36	51.39
	Germinated	33.36	7.29	3.75	3.40	52.20
	Indirectly cooked	26.51	5.99	3.58	3.61	60.31
	Pressure cooked	28.29	7.42	3.37	3.56	57.36
	Solar cooked	27.88	7.37	3.36	3.73	57.66
	Parched	25.51	6.10	3.34	3.72	61.33
'C-235'	Raw	23.00	5.37	3.81	2.47	65.35
	Soaked	24.71	5.47	3.70	2.60	63.52
	Germinated	26.19	5.22	3.59	2.57	62.43
	Indirectly cooked	23.69	5.09	3.45	2.86	64.91
	Pressure cooked	20.49	4.02	2.64	3.17	69.68
	Solar cooked	19.86	3.97	2.50	3.41	70.26
	Parched	21.28	3.68	2.62	3.39	69.03
CD ($P \leq 0.05$)		0.092	0.038	0.039	0.049	1.430
S.E.		0.032	0.013	0.014	0.017	0.050

Proximate composition : The proximate composition of the 'HPG-17' and 'C-235' varieties of chickpea are presented in Table 2. The variations in the protein contents of untreated and treated chickpea varieties were found to be significant. Increases of 2.88 and 7.43%, in the crude protein contents of 'HPG-17' and 'C-235' were observed when subjected to soaking treatment. Almost the same trend was reported by Akinyele and Akinlosotu (1991) who reported that soaking for 4 h and dehulling increased the protein content by 5.7 and 2.7% as compared to values in local brown and local white cowpea cultivars. Germination increased the protein contents in both the varieties with mean values of 33.36 and 26.19%. These however, decreased after indirect cooking. Similarly, Opuku et al. (1981) reported increase in protein content on sprouting ranging from 14 to 40%. The increase in protein content was attributed to loss in dry weight, particularly carbohydrates through respiration during germination. Pressure cooking, solar cooking and parching significantly decreased the levels of protein of chickpea samples to 28.29, 27.88 and 25.51% in 'HPG-17' and 20.49, 19.86 and 21.28% in 'C-235'.

as against the control values of 31.50 and 23.00%, respectively. A decrease in protein content after cooking was reported by Khalil et al. (1986) in field pea, mothbean and pigeonpea. King and Puswastein (1987) reported an increase in the protein content on sprouting of legumes. They attributed this to synthesis of new protein.

The fat contents of the raw samples of 'HPG-17' and 'C-235' were observed as 8.82 and 5.37% respectively. Kasturiba et al (1990) reported the fat contents in chickpea cultivars ranging from 4.61 - 7.79 g per 100 g. The fat content increased after soaking to 8.88 and 5.47%, respectively in both the chickpea varieties. In the germinated and indirectly cooked samples of both the varieties, the fat content was observed to decrease, when compared with raw samples. Pawar and Ingle (1988) observed a decrease in crude fat during germination due to increase in activity of lipases in beans. After pressure cooking and solar cooking, the fat content was found to decrease when compared with the untreated samples. Khalil et al. (1986) reported a decrease in the fat content after cooking, showing that some proportion of fat content was lost during cooking due to leaching. In 'C-235', fat content decreased to 3.68%, when seeds were parched and 6.10% in case of 'HPG-17'. Hira and Chopra (1995) found a decrease in fat content by 11.5% during roasting and dehusking of chickpea seeds. The ash contents in raw seeds of 'HPG-17' and 'C-235' were observed as 4.44 and 3.81%, respectively. Soaking (overnight) of the chickpea seeds decreased the ash contents to 3.96 and 3.70%, respectively. After germination and indirect cooking, the ash contents were found to decrease in both the chickpea cultivars. Lee and Karunanithy (1990) reported losses in total ash contents due to leaching of minerals into soak water on germination. After pressure cooking, solar cooking and parching, the ash contents decreased in both the 'HPG-17' and 'C-235' varieties of chickpea. Sotelo et al. (1987) also reported that cooking diminished the ash content in chickpea variety.

Soaking (overnight) of the chickpea seeds increased the crude fibre contents in both the cultivars. After germination and indirect cooking also, the fibre contents were found to increase in both the chickpea varieties. Giami (1993) reported that germination resulted in an increase in crude fibre content by 4.76%. After pressure cooking and solar cooking, the fibre content were observed as 3.56 and 3.73% in 'HPG-17' and 3.17 and 3.41%, respectively in case of 'C-235'. The parching of the samples of chickpea increased the crude fibre contents to 3.72 and 3.39% respectively in both the varieties.

'HPG-17' variety contained lower contents of carbohydrates (52.00%) when compared with 'C-235' (65.35%). The carbohydrate contents of the untreated and treated samples differed significantly. 'HPG-17' variety also had a lower carbohydrate content when compared with 'C-235' after soaking, pressure cooking, solar cooking and parching. Since the carbohydrate contents were calculated by difference method, the variations in carbohydrate contents in both the varieties were attributed to the differences in the contents of other constituents.

References

Akinyele IO, Akinlosotu A (1991) Effect of soaking, dehulling and fermentation on the oligosaccharides and nutrient content of cowpeas (*Vigna unguiculata*) Food Chem 41 : 43-53

AOAC (1990) Official Methods of Analysis Association of Official Analytical Chemists Washington, DC

FAO (1993) Food and Agricultural Organization FAO Yearbook. Production 1992, Vol. 46, FAO, Rome, Italy, pp 105-115

Giami SY (1993) Effect of processing on the proximate composition and functional properties of cowpea (*Vigna unguiculata*) flour. Food Chem 47 : 153-158

Hira CK, Chopra N (1995) Effect of roasting on protein quality of chickpea (*Cicer arietinum*) and peanut (*Arachis hypogaea*). J Food Sci Technol 32 : 501-503

Kasturiba B, Yamannavar PY, Patil JS, Parameshwarappa R, Surendra HS (1990) Nutritional evaluation of gram (*Cicer arietinum*) Orissa J Agri Res 3 : 64-66

Kataria A, Chauhan MB, Punia D (1989) Antinutrients and protein digestibility (*in vitro*) of mungbean as affected by domestic processing and cooking. Food Chem 32 : 9-13

Khalil JK, Sawaya WN, Al-Mohammad HM (1986) Effects of experimental cooking on the yield and proximate composition of three selected legumes. J Food Sci 41 : 233-235

King RD, Puswastein P (1987) Effect of germination on proximate composition and nutritional quality of winged bean seed. J Sci Food Agri 52 : 104-106

Lee CK, Karunanithy (1990) Effects of germination on the chemical composition of *Glycine* and *Phaseolus* beans. J Sci Food Agri 51 : 437-445

Opuku AR, Ohenhen SO, Ejiofor N (1981) Nutrient composition of millet grains and malts. J Agri Food Chem 29 : 1247

Panse VG, Sukhatme PV (1961) Statistical Methods of Agricultural workers 2nd edn. ICAR, New Delhi 12 : 87

Pawar VD, Ingle VM (1988) Effect of germination on the functional properties of moth beans (*Phaseolus acontifolis* Jacq) flours. J Food Sci Technol 25 : 186-189

Sotelo A, Flores F, Hernandez M (1987) Chemical composition and nutritional value of Mexican varieties of chickpea (*Cicer arietinum*). Qualitas Plantarum - Plant Foods Human Nutri 37 : 299-306

Williams PC, Singh U (1987) Nutritional quality and the evaluation of quality in breeding programmes. In : The Chickpea. Saxena MC, Singh KB (eds) CAB International Oxford, U.K; pp. 329-356

Received 8 March 2000; revised 21 May 2001; accepted 13 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 72-73

Sensitivity of Catheptic Enzymes in Radurized Chicken Meat

S.N. JAMDAR AND P. HARIKUMAR*

Food Technology Division
Bhabha Atomic Research Centre
Mumbai - 400 085, India

Influence of gamma radiation on catheptic enzymes in chicken skeletal muscle has been investigated. The cysteine proteases, cathepsin B and H were found to be more radiosensitive than the aspartic protease, cathepsin D. All the three enzymes showed increased radiosensitivity in the aqueous homogenates than in the tissue. Among the cysteine proteases, cathepsin B was 70% more labile than cathepsin H in the irradiated tissue.

Keywords : Cathepsins, Gamma radiation, Chicken, Muscle proteases.

Application of ionizing radiation is well accepted for the preservation of flesh foods in 'as is' condition (Thayer 1994). Although pathogenic and spoilage microorganisms are eradicated, endogenous proteases especially cathepsins, which are relatively insensitive to ionising radiation, play an important role in determining the quality attributes of radiation processed flesh foods (Ashie et al. 1996). Skeletal muscle possesses a wide spectrum of catheptic enzymes (Harikumar et al. 1974, Huss 1995), which are involved in the post-mortem degradation of muscle proteins. The autolytic products released by these enzymes could facilitate the proliferation of spoilage microorganisms (Huss 1995). Although the structure-function relationships of muscle proteases have received considerable attention (Barrett et al. 1998), their status in radiation processed food systems is poorly understood.

An attempt has been made in the present study, to evaluate the influence of radiation pasteurisation on chicken skeletal muscle cathepsins B, D and H.

Bovine haemoglobin type II, casein, Lα-Arg-β-Naphthylamide [ANA], Nα-benzoyl-DL-Argβ-Naphthylamide [BANA], dimethyl sulfoxide [DMSO], mersalyl acid and fast garnet GBC were obtained from Sigma Chem. Co., St. Louis, USA. All other chemicals were of analytical grade. Chicken muscle was obtained fresh from a local abattoir immediately after slaughter and brought to the laboratory in chilled condition.

Radiation processing : Muscle tissue homogenate was prepared in 0.1 M acetate buffer (pH 3.8) using Polytron Homogeniser at the setting of 5 for 3 min. Chicken muscle tissue and homogenate were separately packed in polythene bags and exposed to gamma radiation [2.5,5.0,7.0 and 15.0 kGY] in Gamma cell at the dose rate of 18 Gy/min under ambient conditions.

Enzyme assays : Activities of cathepsins B, D and H were determined according to Barrett (1972). The assay mixture for cathepsin D consisted of 200 µl 10% homogenate prepared in 0.1 M acetate buffer (pH 3.8), 300 µl buffer solution 0.1 M acetate buffer (pH 3.8) and 500 µl 1% haemoglobin prepared in the same buffer. The reaction was carried out at 50°C for 30 min and stopped by adding 1 ml 10% trichloroacetic acid (TCA). The TCA soluble products were determined using tyrosine as the standard. Activity of cathepsin B was determined using BANA at pH 6.2 at 40°C for 1 h. Cathepsin H was assayed using ANA as substrate at pH 6.8 at 40°C for 30 min. Both the reactions were terminated by the addition of 1 ml mersayl acid. The activities were determined by monitoring the release of β-naphthylamine at 520 nm.

Activities of cathepsins B, D and H in chicken skeletal muscle (Table 1) are comparable to those reported in rat (Kirschke et al. 1983) rabbit (Azanzaj et al. 1979) and fish (Harikumar et al. 1996). Cathepsins B, D and H were inactivated to 95% 18% and 23% respectively in the irradiated (15 kGY) tissue. The corresponding values in tissue homogenate were 95%, 35% and 81% respectively. The accentuated radiolability of the enzymes in the homogenate could be due to elevated levels of free radicals produced by the radiolysis of water, which could inactivate the enzymes (Bhattacharya et al. 2000). Further, the release of enzymes from membrane bound organelles such as lysosomes during homogenisation could also contribute to this phenomenon (Harikumar et al. 1978). Cathepsin B was completely inactivated at 15 kGY in the homogenate as well as in the tissue system. The enhanced lability of this enzyme was also apparent at lower radiation doses (Fig. 1). Enzymes containing cysteine residues are relatively more radiation sensitive due mainly to the oxidation of thiol groups by ^{+}OH radicals (Saha et al. 1996). Being a typical cysteine protease (Katunuma et al. 1981) a

TABLE 1. INACTIVATION OF CATHEPTIC ENZYMES IN CHICKEN SKELETAL MUSCLE BY GAMMA RADIATION (15 kGy)

Enzymes	Specific activity in unirradiated tissue (n moles/h/g tissue) (mean ± s.d.)	Inactivation, %	
		Homogenate	Tissue
Cathepsin B	353 ± 12	95	95
Cathepsin D	13560 ± 22	35	18
Cathepsin H	7225 ± 15	81	23

The values reported are average of 5 independent experiments

*Corresponding Author e-Mail : harikupa@apsara.barc.ernet.in

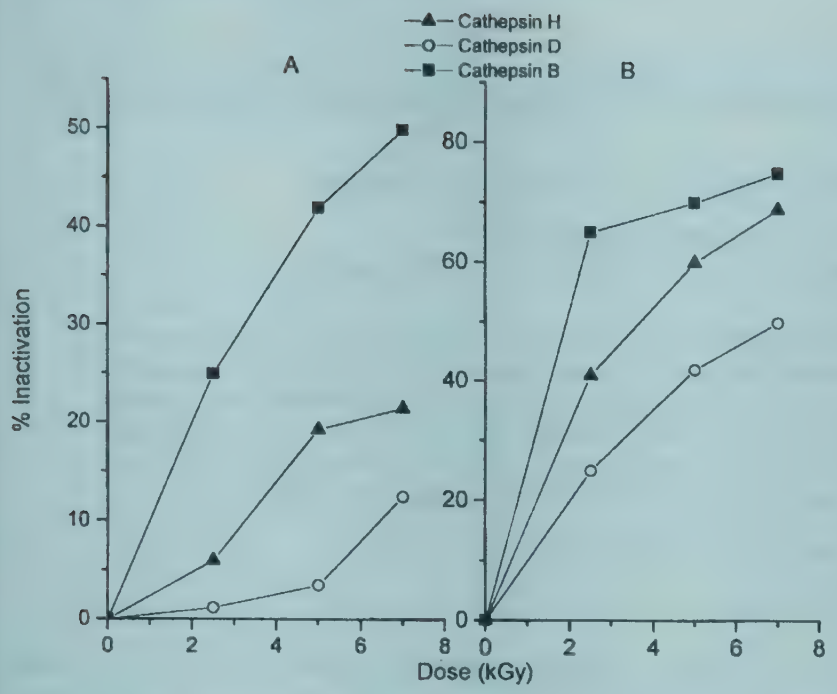

Fig. 1 Radiation sensitivity of cathepsins in chicken skeletal muscle (A) Tissue; (B) Homogenate

similar mechanism might contribute to the radiation sensitivity of cathepsin B also.

This could also explain the differential response of aspartyl (cathepsin D) and cysteine (cathepsins B and H) proteases to radiation (Fig. 1). It is interesting to note that cathepsins B and H, which are known to be similar in structure and kinetics (Fengler and Brandt 1998) show differences in their sensitivity to radiation. For instance, at 7.0 kGY cathepsin B in the tissue (Fig. 1A) was inhibited by 50%, while the activity of cathepsin H was reduced only by 20%. A similar trend was also observed in the tissue homogenate (Fig. 1B). The present results have shown that the radiation processing leads to differential inactivation of catheptic enzymes in chicken meat. While aspartyl proteases such as cathepsin D are relatively unaffected, cathepsin B like enzymes are completely inactivated. These observation point to the need to evolve suitable combination treatments along with radiation for the preservation of meat products.

References

Ashie INA, Simpson BK, Smith JP (1996) Mechanisms for controlling enzymatic reactions in foods. Critical Rev Food Sci Nutr 36: 1-30

Azanzaj L, Raymond J, Robin JM, Cottin P, Ducastaing A (1979) Purification and some physicochemical and enzymatic properties of a calcium ion activated neutral proteinase from rabbit skeletal muscle. Biochem J 183: 339-34.

Barrett AJ (1972) Lysosomal enzymes In: Lysosomes. A laboratory handbook, Dingle JT (ed), NorthHolland Publishing Co, New York, USA

Barrett AJ, Rawlings NO, Woessner JF (1998) In : Hand Book of proteolytic enzymes. Barrett AJ, Rawlings NO, Woessner JF (eds), Acadmic Press, New York

Bhattacharya D, Saha A, Mandal PC (2000) Radiation induced modification of tryptophan and tyrosine residues in flavocytochrome b_2 in dilute aqueous solution. Radiat Phys Chem 59: 71-80

Fengler A, Brandt W (1998) Three dimensional structures of the cysteine proteases cathepsins K and S deduced by knowledge based modelling and active site characteristics. Protein Engng 11: 1007-1013

Harikumar P, Ninjoor V, Kumta US (1974) A method for the evaluation of latency of lysosomal enzymes in chicken skeletal muscle. Die Fleischwirtschaft 54: 936-941

Harikumar P, Brij Bhushan, Warrier SBK, Ninjoor V (1978) Alterations in liver lysosomes induced by whole body x-irradiation of rats. Ind J Biochem Biophys 15: 188-192

Harikumar P, Kakati R, Goswami UC (1996) Vitamin A-deficiency and its effects on the lysosomal enzymes of fish. Internat J Vit Nutr Res 66: 97-100

Huss HH (1995) Postmortem changes in fish In: Quality and Quality Changes in Fresh Fish. Huss HH (ed) FAO Fisheries Technical Paper. pp 35-67

Katunuma N, Towatari T, Kominami E, Hashida S, Takio K, Titani K (1981) Rat liver thiol proteinases: Cathepsin B, Cathepsin H and Cathepsin L. Acta boil med germ. 40: 1419-1425

Kirschke H, Wood L, Roisen FJ, Bird JWC (1983) Activity of lysosomal cysteine proteinase during differentiation of rat skeletal muscle. Biochem J 214: 871-877

Saha A, Mandal PC, Bhattacharyya SN (1996) Radiation induced damage of cysteine residues in dihydroorotate dehydrogenase in dilute aqueous solution. Radiat Phys Chem 48: 61-63

Thayer DW (1994) Wholesomeness of irradiated foods. Food Technol 48: 132-136

Received 3 November 2000; revised 23 May 2001; accepted 13 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 74-75

Indian Soybean Varieties with Low Levels of Lipoxygenase Isozymes

VINEET KUMAR[1], SANJUKTA PATRA[2], ANITA RANI[1] AND S. PRAKASH TIWARI[*1]

[1] National Research Centre for Soybean, Khandwa Road, Indore

[2] School of Life Sciences, Devi Ahilya Vishwa Vidhyalaya (DAVV) - 452 017, India

Lipoxygenase (LOX) isozymes activity, responsible for off-flavour/beany flavour in soy-based products, was comprehensively estimated for the first time in soy-flour of 51 Indian soybean varieties and strains. A large variation for lipoxygenase isozymes activity was found among the Indian cultivars. Lipoxygenase-I varied from 160 to 3192 units/g soy-flours while combined lipoxygenase, II + III ranged from 238 to 1406 units/g of soy-flour. Varieties 'Shilajeet' and 'KhSb-2' possessed the lowest levels of lipoxygenase-I as well as lipoxygenases-II+III. Among indigenous cultivars, 'Pb I' and 'JS 2' were found to be low in lipoxygenase-I as well as lipoxygenase II+III. These varieties can be exploited for food uses, as they will have less beany flavour/off-flavour.

Keywords : Soybean, Fooduses, Off-flavour, Beany flavour, Lipoxygenase, Isozymes, Indian varieties.

Soybean as a food plant, although confined to a marginal region of Himalayas, was known to Indians since ages (Watt 1980). Soybean is endowed with epithet 'functional food of the century' attributed to various health benefits derived from it like cholesterol-free and PUFA (polyunsaturated fatty acids) rich oil which is good for heart patients, lactose-free milk that is suitable for lactose intolerant children, vitamin E as antioxidant and high levels of isoflavones, which can reduce the risk of hazardous diseases like breast cancer, osteoporosis, heart attack, kidney stones and relieves post-menopausal symptoms (Messina 1997). Despite occupying a sizeable area of about 6 million hectares in India, soybean is predominantly utilized as a source of protein in the form of de-oiled cake, which is exported as animal feed, earning huge foreign exchange of about Rs. 20,000 million annually. Although domestic utilization of de-oiled cake has already started in the country, the status of the food uses of soybean stands negligible.

The off-flavour / beany flavour associated with various soy-based foods is the main constraint limiting their utilization. It is well established that the beany flavour results from the formation of volatile carbonyl compounds during the catalysis of polyunsaturated fatty acids and esters containing a cis, cis-1-4-pentadiene system by lipoxygenase (Rackis et al. 1979; Wolf 1975) which constitutes about 1-2% of total seed protein. The soybean seeds contain three lipoxygenase isozymes (Axelrod et al. 1981) which are further grouped into two classes. Class I is characterized by high pH optima of around 9.0 and large amount of 13-hydroperoxides such as lipoxygenase-I (LOX-I), while class II designates pH optima of around 7.0 and formation of equal amounts of 9 and 13 hydroperoxides such as lipoxygenase-II (LOX-II) and III (LOX-III). Genetic elimination of different lipoxygenase isozymes through plant breeding and biotechnological approaches is the ultimate goal for developing and providing soyfood industry with zero or low lipoxygenase as well as to provide household food and nutritional security to Indian population, which suffers from protein under-nutrition. However, the basic information regarding the status of different isozymes of lipoxygenase in Indian soybean cultivars is not available. Variation of lipoxygenases was reported globally in soybean varieties (Omura et al. 1986; Marczy et al. 1991). The present investigation was the first attempt towards identifying Indian soybean varieties with low or zero lipoxygenases.

Fifty one varieties and strains of soybean (Table 1) were sown in the experimental fields of National Research Centre for Soybean, Indore. The seeds were harvested at maturity and the seeds of different varieties were ground in metallic pestle and mortar. The soy-flour obtained was sieved through 150 micron size screen. The soybean extract was prepared by standard method (Suda et al. 1995), which included the homogenization of soy-flour with 100 volumes of phosphate buffer (0.2 M, pH 6.8) in a microtissue homogenizer for 20 min at 0-4^{0}C. The homogenized solution obtained was further centrifuged at 12000 rpm for 10 min at 4^{0}C. The supernatant so obtained was used as crude extract for assaying lipoxygenases. Quantitative determination of lipoxygenases was carried out following the standard method (Axelrod et al. 1981) with slight modification. The reaction mixture for assaying lipoxygenase-I consisted of crude extract as the source of enzyme, boric acid-borax buffer (0.2 M, pH 9.0) and 10 mM sodium linoleate. Lipoxygenase isozymes II + III were analysed collectively with the reaction mixture consisting of crude extract, phosphate buffer (0.2 M, pH 6.8) and 10 mM sodium linoleate as a substrate. The change in absorbance was recorded in Shimadzu UV-160A spectrophotometer at 234 nm. One unit of enzyme activity was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 per min at 234 nm.

A large variation for lipoxygenases activity was found among the cultivars tested in the present investigation. LOX-I activity varied from 160 units/g of soy-flour of variety 'Shilajeet' to 3192 units/g of soy flour of variety 'NRC 2' (Table 1). LOX II + III collectively varied from 238 units/g of soyflour as observed in case of 'Shilajeet' to 1406 units/g of soy-flour of variety 'Co-1'. It is to be noted that the variety 'Shilajeet' desirably possessed the lowest activity of LOX-I as well as the lowest combined activity of LOX-II + III.

The traditional indigenous material or varieties originating from them viz 'JS 2', 'Kalitur', 'Pb I', 'T49' showed interesting

*Corresponding Author E-mail : nrcsoya@hubl.nic.in

TABLE 1. LIPOXYGENASE ISOZYME ACTIVITIES* IN INDIAN SOYBEAN VARIETIES/STRAINS

Variety	LOX-I	LOX-II + III
'Alankar'	2500	1080
'Ankur'	1340	372
'Co 1'	2332	1406
'Durga'	2524	1258
'Gaurav'	2484	906
'GS 2'	960	424
'Hardee'	1858	400
'Harit Soya'	1260	550
'Improved Pelican'	3064	896
'JS 335'	1824	494
'JS 71-05'	1200	754
'JS 76-205'	2272	1124
'JS 79-81'	2488	830
'JS 90-41'	3008	456
'JS2'	996	438
'Kalitur'	1854	439
'KhSb 2'	492	292
'Lee'	2648	1008
'MAUS 1'	2412	736
'MACS 13'	3008	1106
'MACS 450'	1442	840
'MACS 57'	2496	1062
'MACS 58'	708	474
'MACS 124'	1120	576
'MAUS 2'	2152	882
'Monetta'	2464	783
'NRC 37'	1632	636
'NRC 38'	1516	584
'NRC 41'	1680	812
'NRC 7'	1228	354
'NRC 12'	1876	497
'NRC 2'	3192	793
'Pb 1'	818	375
'PK 262'	2216	600
'PK 472'	2930	757
'PK 564'	976	862
'PK 1029'	1640	642
'PK 327'	1472	776
'PK 471'	2100	856
'Pusa 16'	1308	414
'Pusa 22'	1260	1002
'Pusa 35'	1196	422
'Pusa 40'	2416	762
'Samrat'	1210	528
'Shilajeet'	160	238
'Shivalik'	1852	838
'SPT 88'	2392	906
'T 49'	1646	693
'VLS 21'	1668	607
'VLS 2'	1056	761
'VLS 47'	1094	582

*Units per gram of soy-flour where one unit was taken as equivalent to amount of enzyme that generated change in absorbance 1.0 per minute at 234 nm

	CD at 5%
Lipoxygenase I (LOX-I)	69
Lipoxygenase II+III (LOX-II+III)	42

results. The variety 'Pb I' showed lowest LOX-I (818 units/g soyflour) activity and LOX-II + III (375 units/g soyflour) activity, followed by 'JS 2' showing LOX-I of 996 units/g soyflour and LOX II + III of 438 units/g of soy-flour (Table I). Thus, among the indigenous varieties, 'Pb I' and 'JS 2' (selections from old and indigenous Tehri-Garhwal material) were found to be low in lipoxygenases-I as well as combined lipoxygenase-II + III.

To conclude, 'Shilajeet', 'KhSb2', 'Pb I', 'GS 2', 'PK 546' and 'JS 2' were identified as the varieties possessing low levels of LOX-I whereas 'Shilajeet', 'KhSb-2', 'NRC 7', 'Ankur', 'Pb I' and 'Hardee' possessed low levels of LOX II + III. 'Shilajeet' and 'KhSb 2', with low levels of all the three lipoxygenases i.e. LOX-I as well as LOX II + III were identified as ideal for food uses. However, these two varieties are not in the seed-chain presently. Efforts should be made to make the seeds of these two varieties available to end-users through a special seed multiplication programme. The soyfoods prepared from the seeds of these identified varieties will have less degree of off-flavour / beany flavour, thus imparting a wide acceptance and augmented utilization of soy-based products in the country to the benefit of millions of mal-nourished and under-nourished population. It will also help in reducing the foreign-dependence in the volatile post-WTO scenario by creating a domestic market for Indian deoiled cake the export of which is, hitherto, subjected to fluctuation in the international trade.

References

Axelrod B, Cheesebrough TM, Lackso S (1981) Lipoxygenases in soybeans. Methods Enzymol 71: 441-451

Marczy JS, Simon ML, Moz SiL, Szajani B (1995) Comparative study on the lipoxygenase activities of some soybean cultivars. J Food Chem 43 : 313-315

Messina Mark J (1997) Soyfoods; Their role in disease prevention and treatment In : Soybean-Chemistry, Technology and Utilization : Liu Keshun (ed) Chapman and Hall, New York. pp 443-447

Omura Y, Akiyamo Y, Saio KD (1986) Distribution and varietal differences of lipoxygenase in soybean seeds. Nippon Shokuhn Kogro Gakkaiishi 33 : 653-658

Rackis JJ, Sessa DJ, Honig DH (1979) Flavour problems of vegetable food protein. J Am Oil Chem Soc 56 : 262-271

Suda I, Hajika M, Nashiba Y, Furuta S, Igita K (1995) Simple and rapid method for the selective detection of individual lipoxygenase enzymes in soybean seeds. J Agric Food Chem 43 : 742-747

Watt G (1980) in A Dictionary of Economic Products of India. Volume III Cosmos Publication Delhi, India. pp 509-511

Wolf WJ (1975) Lipoxygenase and flavour of soybean protein products. J Agric Food Chem 23 : 136-141

Received 18 September 2000; revised 30 May 2001; accepted 13 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 76-78

Utilisation of Peel in Plantain Wine Production

R. ALI-SIMON AND N. BADRIE*

Department of Food Production,
Faculty of Agriculture and Natural Sciences,
University of the West Indies,
St. Augustine, Trinidad and Tobago, West Indies.

Wines were produced from plantain (*Eumusa*), with addition of no peel (T_1), 6% peel (T_2), 6% peel with 20% passion fruit concentrate (T_3), 12% peel (T_4) and 12% peel with 20% passion fruit concentrate (T_5). The must was adjusted to 3.1 - 3.5 with 22 - 23^0 Brix. The tannin content increased on addition of peel from 16.25 mg/100 ml to 23.37 mg/100 ml. Addition of passion fruit concentrate further increased the tannin content to 27.7 ± 0.40 mg/100 ml. The total soluble solids and alcohol content of wines varied between 8.0 - 9.0^0 Brix and 13.0 - 14.7% respectively. Wine treatments were not different ($P > 0.05$) sensory attributes. Clarity was the most acceptable attribute in wines, while taste was least liked. Overall scores indicated that all wines were neither liked nor disliked to liked slightly.

Keywords : Plantain peel, Passion fruit, Wines, Tannin, Sensory attributes.

Plantain fruits like bananas belong to the genus *Eumusa* (Purseglove 1972) which, unlike bananas are non-edible when raw and thus, need to be processed. Ketiku (1973) reported that plantains consist of water (66.4 g/100 g), carbohydrate (31.2 g/100 g of which 66% starch and 17% sugar), proteins (1.1 g/100 g), fat (0.4 g/100 g) and ash (0.9 g/100 g). In the Caribbean, plantains are boiled or fried and eaten as chips. Sharrock (1996), mentioned that a banana beer low in alcohol content and short shelflife was made from the juice of ripe bananas in several parts of Africa. Banana wines that are not fortified with sugars are weak and have a short shelflife (Simmonds 1966). Kordylas (1990) reported that the peel and pulp of ripe bananas contain appreciable amounts of fermentable sugars that can be used for alcoholic fermentation.

Fruit wines refer to the fermented juice of fruits other than grapes (Cariri 1992; Pilgrim 1994). Fermentation is a process in which yeasts convert the sugars in the fruit juice into alcohol, carbon dioxide and small amounts of various by-products (Cariri 1992). As the plantains are perishable, the objectives of the investigation were to utilise both the plantain pulp and peel in the production of wines and find out the effects of peel with or without passion fruit additions on quality attributes.

Mature, firm and yellow French plantains (*Eumusa* AAB gp) were purchased from a local supermarket. The fruits were stored for 5 days at room temperature (28^{0}C) until they were overripe. Passion fruit (*Passiflora edulis* var. flavicarpa) concentrate of 36^0 Brix was kept frozen at -18^{0}C and blended with plantain *puree* in some wine treatments. Wine yeast, *Saccharomyces cerevisiae,* (Formula 67 Plus, Unicam Foods Ltd., Norwich, England) a rapid starting, high alcohol tolerance, natural flavour enhancement, quick settling and fast clearing yeast was used for fermentation. Also, a compound of diammonium phosphate with ammonium phosphate was used for the stimulation of the yeasts in fermentation. Fig 1 shows various steps in the production of plantain wines. The fruits were washed and sanitised (100 ppm chlorine). The plantain

*Corresponding Author

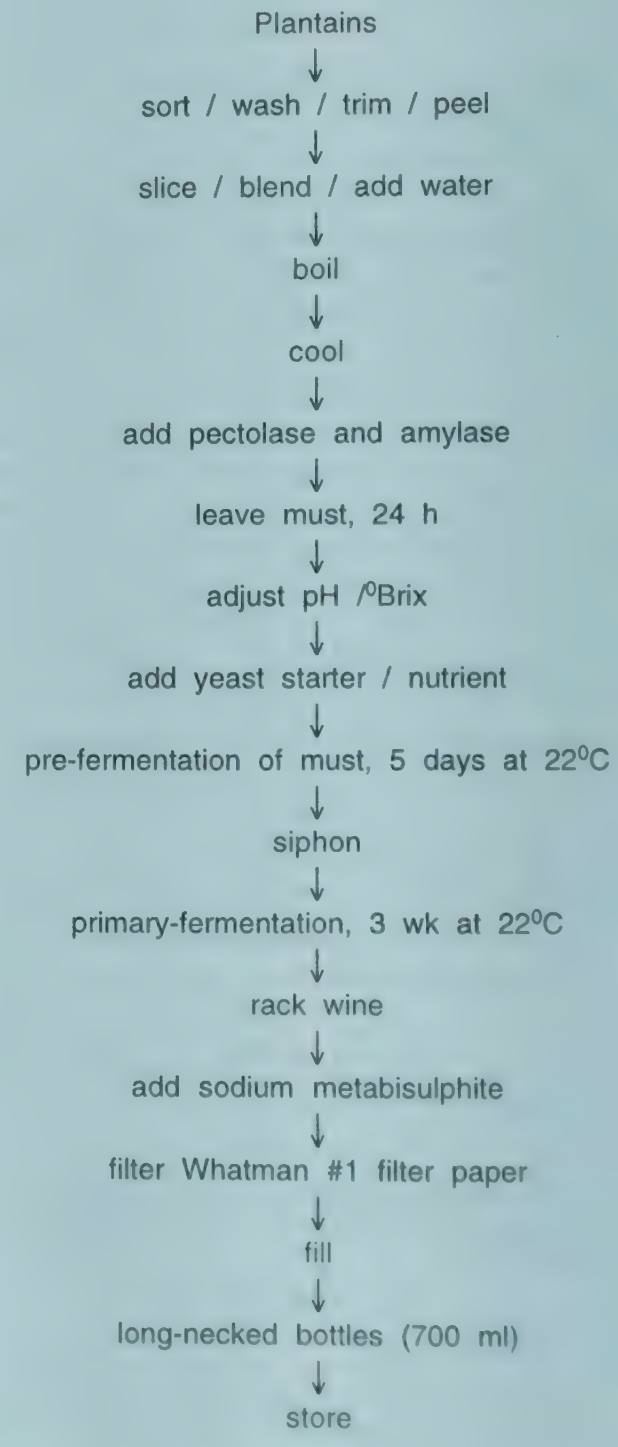

Fig. 1 Fermentation steps for plantain wine production

was sliced and blended for 1 min (Oster model #889 16R, Schaumburg IL). Pre-fermentation was performed in 7-litre food grade plastic buckets, while primary fermentation was in 4.5 litre glass demijohn bottles equipped with rubber bungs incorporating air locks filled with 60 ppm sodium metabisulphite. All plantain wine formulations consisted of plantain pulp with or without peel (24%), water (36%), granulated sucrose (~37.5 ± 1.0%), pectolase (0.15%), amylase (0.03%), yeast (0.23%), compound diammonium phosphate with ammonium phosphate (2.2%), and sodium metabisulphite (0.15%). Wine treatments consisted of plantain pulp with no inclusion of peel (T_1), 6% peel without addition of passion fruit concentrate (T_2) or with addition of passion fruit concentrate (T_2), 12% peel without the addition of passion fruit concentrate (T_4) or with passion fruit concentrate (T_5).

The pH of the must was measured using an Expandable Ion Analyzer EA 920 (Orion Research Inc.) and adjusted to 3.1 - 3.5 by the addition of citric acid using method #962.12 (AOAC 1990). A calculated quantity of crystalline sucrose was added to the 24 h must to obtain a total soluble solid content 22 - 23^0 Brix by using the formula of Cooke and Lapsley (1988). Each treatment was replicated.

The total soluble solids (^{0}Brix) of the must and the wine were measured on a Leica, hand-held temperature compensated, hand-held refractometer (Atago E Type Series, Leica Inc., Buffalo, N.Y.). The total titratable acidity (TTA) expressed as % citric acid of the must and wines was analysed using the method #962.12, (AOAC 1990). The specific gravities of the must and wines was checked using a Stevenson hydrometer (Peter Stevenson Ltd) and the alcohol content was calculated as outlined by Cariri (1992). Colour intensity (% light absorbance) of the final wines was measured by using a Lambda 3B UV/VIS spectrophotometer (Perkins Elmer Inc, Altamira, San Juan, Puerto Rico) at 475 nm. The spectrophotometer was standardized at 100% transmittance using distilled water. The tannin content of wines was determined using the method # 952.03 (AOAC 1990).

Appropriate dilutions of three samples (duplicate) were made on the must (after 24 h) and wines (after 2 weeks of fermentation and storage) for the following counts : total plate count on plate count agar incubated at 37^{0}C for 48 h, yeast count and mold count on potato dextrose agar acidified with sterile 10% tartaric acid at 25^{0}C for 3 - 5 days as described in the Compendium of Methods for the Microbiological Examination of Foods (APHA 1992). Gram staining was performed as outlined by Benson (1990) for the identification of the microorganisms.

Sensory analysis was conducted in the Food Biology Laboratory, Department of Food Production, University of the West Indies, Trinidad, West Indies. Quality attributes of clarity, colour, odour or taste with astringency were evaluated, based on a 5 point Hedonic scale of 1-liked the most; 2-liked slightly; 3-neither liked nor disliked; 4-disliked slightly and 5-disliked the most. Details of the test were explained to the panelists prior to sensory evaluation. Wine samples (20 ml) were coded (3-digit), chilled (5^{0}C) and served in long stemmed, wide mouthed, wine glasses with water, cheese and crackers. The panel in each triplicate session consisted of 15-18 persons who were familiar with sensory evaluation and were wine drinkers. Analysis of variance (5% level of significance) was performed to investigate the effects of treatments on the Hedonic scores using the method of Watts et al (1989).

TABLE 1. PHYSICO-CHEMICAL CONSTITUENTS OF WINE TREATMENTS

Treatments	TSS, ^{0}Brix	Alcohol, % v/v	Light absorbance, %	% TA, mg/100 ml, as CA	Tannin, mg/100 ml
T_1	9.0	13.0	3.50	13.2	16.25
T_2	8.5	14.1	6.95	14.7	21.10
T_3	8.5	14.7	7.06	13.4	25.97
T_4	8.0	13.7	7.40	14.3	23.37
T_5	8.5	14.4	13.50	14.1	27.25
SE	0.5	0.5	0.02	0.4	1.10

TSS - Total soluble solids; TA - titratable acidity; CA - citric acid T_1 - plantain + 0% peel; T_2 - plantain + 6% peel; T_3 - plantain + 6% peel + passion fruit; T_4 - plantain + 12% peel; T_5 - plantain + 12% peel + passion fruit

Table 1 indicates the results of the physico-chemical analyses by variation of various treatments. All treatments had a specific gravity < 1.00, varying between 0.992 and 0.994 and had < 9.0^0 Brix, which were considered to be dry according to Cariri (1992). The alcohol content varied between 13.0 and 14.7%. The pH of all the wines varied between 3.2 - 3.4 and had total titratable acidity of 13.2 - 14.7 mg/100 ml. Citric acid has been reported to be the major acid in plantains. The tannin content (mg/100ml) of wines increased with the addition of peel and was further increased by the addition of passion fruit T_3 and T_5 had higher tannin content (Table 1). The presence of tannins in plantain tissues are responsible for astringency (Simmonds 1966; Seymour 1993). According to Sahni et al. (1998) polyphenol oxidase can catalyse oxidation of polyphenol compounds in fruits to quinones, which then polymerise to dark melanine pigments of unknown structures such as formation of brown or black spots from chloregenic acid in bananas. On further addition of peel, the intensity of colour increased as evident by the increase in light absorbance (Table 1). Addition of passion fruit, further increased the colour intensity, which may be related to the pigment content of passion fruit (Table 1). Results in Table 1 indicate that wines of 12% peel without passion fruit (T_4) and with passion fruit (T_5) had 7.40% and 13.50% light absorbance, respectively, which were higher than wine treatments with 6% peel either without passion fruit (T_2) or with passion fruit (T_3). Gomez and Matill (1949), have indicated that plantain like the banana is relatively rich in ascorbic acid (15-18 mg/100 g pulp) and considerably higher in carotene content than banana. Norepinephrine, 5-hydroxytryptamine and dopamine are present in plantains and bananas (Foy and Parratt 1960).

There were no significant differences in treatments for sensory scores. The overall scores obtained indicated that all treatments were neither liked nor disliked to liked slightly

(2-like slightly, 3-neither liked nor disliked : T_1 : 2.07; T_2 : 2.22; T_3 : 2.20; T_4 : 2.07; T_5 : 2.16; SE ± 0.15). Generally, in all wine treatments, clarity was given the most acceptable scores of 1.40 - 1.98, being liked slightly to liked. the most, followed by colour of 1.83 - 2.06 neither liked nor disliked to liked the most, odour 2.13 - 2.53 neither liked nor disliked to liked to liked slightly and taste 2.78 - 2.80 neither liked nor disliked nor disliked to liked slightly. Of all the attributes, taste was given the least desirable score. General comments by panelists did indicate that the wines were astringent. Tannins interact with the salivary proteins and glycoproteins resulting in astringent taste (Haslam 1981).

The average plate counts of yeast colonies in must (after 24 h) were in the range 4.5 - 8.1 x 10^2 cfu/ml and total plate counts of 2.0 - 5.0 x 10^3 cfu/ml. After 2 weeks of fermentation, the total number of microorganisms increased but did not vary ($P > 0.05$) in the various treatments having a range of 2.9 x 10^4 - 9.7 x 10^6 cfu/ml. All wines stored at 22^{0}C for 2 weeks had total counts of < 10 - 65 cfu/ml. The low population of microorganisms could be related to the addition of sodium metabisulphite (Fig. 1) and alcohol content (Table 1). Amerine et al. (1980) reported that in the absence of other ways to control microbial growth, wines with less than 10% alcohol tended to spoil more easily than those containing over 10% alcohol.

References

Amerine M, Berg H, Kunkee R, Ough C, Singleton V, Webb A (1980) Technology of Wine Making. AVI Publishing Co, Westport, Connecticut, USA

AOAC (1990) Official Methods of Analysis, Vol 2. Association of Official Analytical Chemists, Virginia, USA

APHA (1992) In : Compendium of Methods for the Microbiological Examination of Foods, Vanderzant C., Splittoesser DF (eds.). American Public Health Association, Washington, DC pp 75-87 : 239-249

Benson HJ (1990) Microbiological Applications : A Laboratory Manual in General Microbiology. Wm. C. Brown Publishers, IA 56-58

Cariri (1992) Basic Principles in Fruit Wine Production. Caribbean Industrial Research Institute, The University of the West Indies, St. Augustine campus, Trinidad, WI

Cooke GM, Lapsley JT (1988) Making Table Wine at Home University of California, Davis, California.

Foy JM, Parratt JR (1960) Noradrenaline and 5-hydroxytryptamine in plantain. J Pharm Pharmacol 12 : 360-364

Gomez A, Mattill HA (1949) Ascorbic acid and carotene content of plantain. Food Research 14 : 177-181

Ketiku AO (1973) Chemical composition of unripe (green) and ripe plantain (*Musa paradisiaca*) J Sci Food Agric 24 : 703-707

Kordylas JM (1990) Alcoholic fermentation of fruit juices to wines. In : Processing and Preservation of Tropical and Subtropical Foods. Education Low Priced Books, Hampshire, England

Haslam E (1981) Vegetable tannins. In : Biochemistry of Plants Vol. 7, Conn EE (ed.) Academic Press, New York

Pilgrim R (1994) Post-harvest handling in minor exotics. In : Third Regional Workshop on Tropical Fruits. Inter-American Institute for Cooperation on Agriculture (IICA), Grenada

Purseglove JW (1994) Tropical Crops : Monocotyledons. Vols 1 and 2. Longman Group Ltd., Essex England

Seymour GB (1993) Banana In : Biochemistry of Fruit Ripening Chapman and Hall, Taylor JE, Tucker GA (eds.) London pp 83-106

Sahni CK, Kumar S, Kapoor HC (1998) Browning reaction of foods - Recent approaches to control browning - An overview. Indian Food Packer 52(4) : 40-47

Sharrock S (1996) Uses of *Musa* In : Networking banana and plantain Annual report. International Network for the Improvement of Banana and Plantain (INIBAP), France

Simmonds NW (1966) Banana. Longman, London, UK pp 252-275

Watts BM, Ylimaki GD, Jeffery LE, Elias LG (1989) Basic Sensory Methods for Food Evaluation. International Developmental Research Centre, Ottawa, Canada, pp 60-79

Received 28 March 2000; revised 30 May 2001; accepted 13 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 79-81

Microbiological Status of Buffalo Meat from Slaughterhouses and Retail Outlets

ARUN KUMAR TIWARI, S.P. SINGH* AND ARUN KUMAR

Department of Veterinary Public Health,
College of Veterinary Sciences,
G.B. Pant University of Agriculture and Technology, Pantnagar - 263 145, India.

A study was conducted to evaluate the sanitary quality of buffalo meat produced in municipal slaughterhouses and sold at the retail outlets located in Bareilly, Haldwani and Kichha. Meat samples (103) collected were subjected to standard plate count (SPC), Staphylococcal, coliform, psychrophillic and yeast and mould counts. It was found that SPC (log cfu/g) of buffalo meat samples collected from slaughterhouses and retail shops varied from 6.3 to 6.7 and 7.0 to 7.9, respectively. Staphylococcal counts (log cfu/g) of the same samples were in the range of 4.8 to 5.4 in slaughterhouses and 5.3 to 6.5 in retail shops samples. Coliform counts varied from 3.6 to 4.4 and 3.6 to 4.5 in slaughterhouses and retails shops samples, respectively. The samples collected from slaughterhouses showed psychrophillic counts ranging from 3.6 to 4.4 and from retail shops 4.2 to 5.8. Yeast and mould counts of the samples obtained from slaughterhouses and retail shops ranged from 3.5 to 4.8 and 4.6 to 5.6, respectively.

Keywords : Meat, Microbial quality, Slaughterhouses, Retail shop, Buffalo meat.

The total meat production of India is approximately 3.41 million metric tonnes (FAO 1997), of which major portion comes from buffalo meat (1.40 million metric tonnes). However, India contributes less than 1% of total meat exported throughout the world. The poor sanitary quality of meat is one of the main reasons for the lower demand of the meat from Indian processors in world market.

Since the meat is an excellent medium for the growth of the microorganisms, poor hygienic procedures and inadequate storage conditions provide ample opportunity for their growth. During slaughtering, dressing and further processing, meat surface invariably comes in contact with contaminated tools, floor and other equipment and get easily contaminated (Narsimha Rao and Ramesh 1988).

Several workers have attempted to establish the microbial standards of meat food (Pivnick et al. 1976; Bachhil 2000) and *Coliform, Staphylococci, Clostridium perfringens, Salmonellae, Pseudomonas, Enterococci* and yeast and moulds have been used to assess the microbial safety and sanitary conditions during slaughtering and processing of meat foods (Tompkin 1983). The present study was undertaken to evaluate the microbial quality of buffalo meat produced at slaughterhouses of Bareilly, Haldwani and Kichha and marketed at retail outlets in the respective localities.

Meat samples (103) weighing approximately 30 g each were collected aseptically from pre-scapular region of buffalo carcasses slaughtered during early morning hours at the slaughterhouses and also from the retail shops located at Bareilly (40), Haldwani (30) and Kichha (33). The samples were brought to laboratory under chilled condition and were cut into small pieces after trimming of fascia and 10 g sample was analyzed for microbial load.

Microbiological examination of meat samples : The meat samples collected as above were examined for SPC, coliform, staphylococcal, psychrophillic and yeast and mould counts. The SPC of meat was estimated as per the procedure described in APHA (1976). Staphylococcal, psychrophillic and yeast and mould counts were done according to methods described earlier. (Thatcher and Clark 1978; Tibin and Melton 1990)

Statistical analysis : The data on microbial counts obtained from meat samples were subjected to statistical analysis. The various counts from slaughterhouses and retail shops were compared by using the analysis of variance (Snedecor and Cochron 1968).

Standard plate count : Mean values of SPC (log cfu/g) of meat samples collected from the slaughterhouses of Bareilly, Haldwani and Kichha were recorded to be 6.7, 6.4 and 6.3, respectively (Table 1). The counts were almost similar to those recorded by earlier workers in meat samples (Rao and Rao 1983; Narsimha Rao and Ramesh 1988; Barbuti et al. 1992). The SPC was significantly higher ($P < 0.05$) in Bareilly than those of Kichha slaughterhouse samples and the SPC of meat samples from Haldwani slaughterhouse were lower than those of Bareilly but higher than those of Kichha slaughterhouse. However, the difference was non-significant ($P > 0.05$). The SPC (log cfu/g) of meat samples collected from retail shops located in Bareilly, Haldwani and Kichha showed mean values of 7.9, 7.3 and 7.0, respectively (Table 1). The counts recorded were very close to the counts obtained by Borah et al. (1992). However, these counts were higher than the standard proposed by Pivnick et al. (1976) and Bachhil (2000). Such high counts in meat samples could be due to faulty transport practices and poor hygienic conditions maintained at retail shops. On statistical analysis, it was found that the meat samples collected from retail shops showed significantly higher counts than the corresponding slaughterhouses in Bareilly ($P < 0.01$), Haldwani and Kichha ($P < 0.05$).

**Corresponding Author*

TABLE 1. COMPARISON BETWEEN THE MEAN MICROBIAL COUNTS PER GRAM OF BUFFALO MEAT SAMPLES OF SLAUGHTERHOUSES AND RETAIL SHOPS.

Location	SPC	Coliform count	Staphylococcal count	Psychrophilic count	Yeast and Mould count
Bareilly					
Slaughterhouse	5.8×10^6 (6.7 $\log_{10}$)	2.8×10^4 (4.4 $\log_{10}$)	2.8×10^5 (5.4 $\log_{10}$)	2.8×10^4 (4.4 $\log_{10}$)	6.6×10^4 (4.8 $\log_{10}$)
Retail shops	8.7×10^7 (7.9 $\log_{10}$)**	3.3×10^4 (4.5 $\log_{10}$)	3.5×10^6 (6.5 $\log_{10}$)**	6.7×10^5 (5.8 $\log_{10}$)*	4.6×10^5 (5.6 $\log_{10}$)**
Haldwani					
Slaughterhouse	2.6×10^6 (6.4 $\log_{10}$)	4.5×10^3 (3.6 $\log_{10}$)	8.6×10^4 (4.9 $\log_{10}$)	4.2×10^3 (3.6 $\log_{10}$)	1.0×10^4 (4.0 $\log_{10}$)
Retail shops	2.3×10^7 (7.3 $\log_{10}$)**	6.5×10^3 (3.8 $\log_{10}$)	2.2×10^5 (5.3 $\log_{10}$)	1.2×10^5 (5.0 $\log_{10}$)*	4.5×10^4 (4.6 $\log_{10}$)
Kichha					
Slaughterhouse	1.9×10^6 (6.3 $\log_{10}$)	7.9×10^3 (3.9 $\log_{10}$)	7.8×10^4 (4.8 $\log_{10}$)	9.2×10^3 (3.9 $\log_{10}$)	3.5×10^3 (3.5 $\log_{10}$)
Retail shops	1.2×10^7 (7.0 $\log_{10}$)*	4.1×10^3 (3.6 $\log_{10}$)	2.6×10^5 (5.3 $\log_{10}$)*	1.8×10^4 (4.2 $\log_{10}$)*	1.0×10^5 (5.0 $\log_{10}$)**

* Indicates significantly higher counts in retail shops than the slaughterhouse samples at $P < 0.05$

** Indicates significantly higher counts in retail shops than the slaughterhouse samples at $P < 0.01$

All the meat samples examined were found to be positive for the presence of coliforms. The mean coliform counts (log cfu/g) of meat samples collected from slaughterhouses were 4.4, 3.6 and 3.9 in Bareilly, Haldwani and Kichha, respectively (Table 1). These counts although lower than the counts recorded by Park (1971) were higher than the standard recommended by ISO (1979). The high count recorded in the samples could be associated with unhygienic conditions prevailing in the slaughterhouses and its environment. The difference between Bareilly and Haldwani was found to be significant ($P < 0.05$). Such a high count in Bareilly could be either due to the large number of animals slaughtered with inadequate floor space, leading to overcrowding, thereby increasing the chances of contamination of inadequate and unchlorinated water supplied to the slaughterhouses. However, the differences in the counts between Bareilly and Kichha samples as well as between Haldwani and Kichha sample were non-significant ($P > 0.05$).

The mean coliform counts (log cfu/g) in meat samples obtained from retail outlets of Bareilly, Haldwani and Kichha were 4.5, 3.8 and 3.6, respectively (Table 1). The coliform counts in meat samples from retail shops did not show any significant increase when compared with the samples of the respective slaughterhouses. Park (1971) also recorded similar trends in his findings. However, these counts were higher than

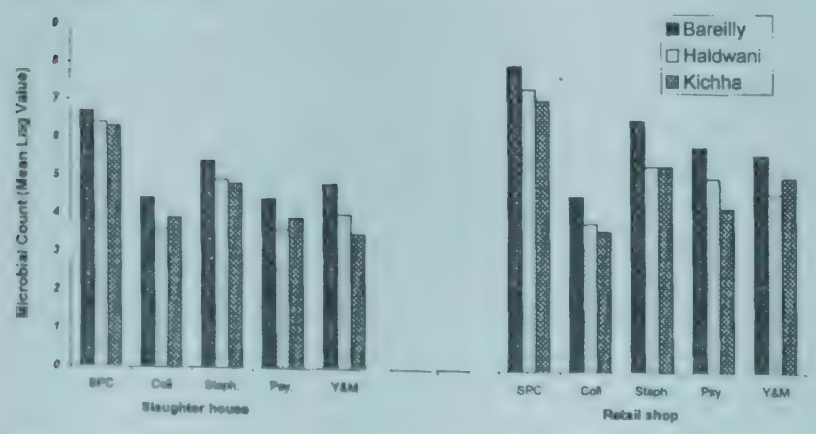

Fig. 1. Comparison of mean log values of microbial counts/g of meat in slaughterhouse and retail shop samples

the ISO (1979) standards, indicating poor sanitary quality of meat samples.

The samples were found to be contaminated with *staphylococci* with the mean count of 5.4 (Bareilly), 4.9 (Haldwani) and 4.8 (Kichha). The counts recorded in the present study were higher than the counts reported by earlier workers (Pivnick et al. 1976; Foster et al. 1977; Scriven and Singh 1986). The unsatisfactory personal hygiene of slaughterhouse workers, their knives, hides/skins of animal and the floor contents could have contributed to the increased number of organisms in the meat samples. When compared, significantly higher counts were noticed (Table 1, Fig. 1) in Bareilly than Haldwani ($P < 0.05$) and Kichha ($P < 0.01$), although the difference between counts from Haldwani and Kichha were non-significant ($P > 0.05$).

Staphylococcal counts in the meat samples collected from retail shops showed mean values of 6.5, 5.3 and 5.3 in Bareilly, Haldwani and Kichha, respectively (Table 1).

The increased counts in retail shops could be attributed to the factors associated with the transport system and environmental conditions causing contamination and providing an opportunity to the organisms to grow, as meat produced at slaughterhouses are generally distributed through uncovered vehicles like tempo, tanga, and rickshaw.

The mean counts of psychrophils (log cfu/g) in meat samples obtained from Bareilly, Haldwani and Kichha slaughterhouses were recorded to be 4.4, 3.6 and 3.9, respectively (Table 1). Psychrophillic counts of meat samples of Bareilly slaughterhouse revealed significantly high counts as compared to the counts of other two slaughterhouses ($P < 0.5$). However, comparison of counts between Haldwani and Kichha indicated a non-significant ($P > 0.05$) difference (Table 1). These counts were higher than the respective slaughterhouses (Fig. 1). The increases in the counts of retail shop samples of Bareilly and Haldwani were found to be significant ($P < 0.05$) when compared to the meat samples of the slaughterhouses of the same localities. These increase in the counts may be attributed to the poor hygienic condition and improper storage

facilities at retail shops.

Like other counts, attempts were also made to count the yeast and moulds of the meat samples. The estimated mean values of yeast and mould counts per gram were found to be 4.8 $\log_{10}$ 4.0 $\log_{10}$ and 3.5 $\log_{10}$ in Bareilly, Haldwani and Kichha slaughterhouses, respectively (Table 1). These values were in accordance with the findings of earlier workers (Simard and Auclair 1981).

It was observed that the yeast and mould counts were highest in Bareilly as against the other slaughterhouses (Fig. 1). Yeast and moulds being environmental contaminants, usually contaminate the carcasses at the time of cutting and dressing. High yeast and mould counts in Bareilly could be due to slaughtering operation being done in open air. The difference between the counts of meat samples from Haldwani and Kichha slaughterhouses were found to be non-significant ($P > 0.05$).

The mean yeast and mould counts in the meat samples of retail shops were recorded to be 5.6 $\log_{10}$, 4.6 $\log_{10}$ and 5.0 $\log_{10}$ in Bareilly, Haldwani and Kichha, respectively (Table 1). The counts observed in the present study were higher than the counts reported by Simard and Auclair (1981), Scriven and Singh (1986). Higher counts recorded in the present study could be associated with the poor hygienic standards in and around the slaughterhouse and retail shops.

Conclusion

The results obtained in the present study indicated that the sanitary quality of buffalo meat produced at slaughterhouses and sold at retail shops was unsatisfactory. It is imperative that slaughterhouse environment under which the meat is produced and transported to the retail outlets needs proper care and attention.

The authors thank the Dean, College of Veterinary Sciences, for providing necessary facilities to conduct the present study.

References

APHA (1976) Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington pp 277-298

Bachhil VN (2000) Standards for Quality Control of Meat and Meat Products. In : Proceedings of Workshop on Meat Inspection / Hygiene and Slaughter house Management, G. B. Pant University of Agriculture and Technology, Pantnagar. pp 181-189

Barbuti S, Manganeili E, Ghisi M, Campanini M (1992) Microbiological characteristics of pig carcasses in Italian slaughterhouses. In : Proceedings of the 38th International Congress of Meat Sciences and Technol. Clermont-Ferrand, France. 4 : 617-620

Borah P, Patgiri GP, Boro BR (1992) Bacteriological quality of market pork in Guwahati City. Indian Vet J 69(9) : 773-775

FAO (1997) Food and Agriculture Organization of the United Nations, Rome, Italy Vol. 51

Foster FJ, Fowler JL, Ladeis WJ (1977) A bacteriologist survey of raw ground beef. J Food Prot. 42(11) : 790-794

ISO (1979) International Organization for Standardization for meat products : Detection and enumeration of presumptive coliform bacteria and presumptive *Escherichia coli.* ISO 3811 p 5

Narshimha Rao D, Ramesh BS (1988) Microbial profile of minced meat. Meat Sci 23(4) : 279-291

Park KS (1971) Bacterial contamination of meat during transport from the butchery to the home. Korean J Public Health 8(1) : 21-26

Pivnick H, Erdman IE, Thomson DC, Roberts G, Johnston MA, Conley DR, Lachepelle G, Purvis UT, Foster R, Milling M (1976) Proposed microbiological standards for ground beefbased on Canadian Survey. J Milk Food Technol 39(6) : 408-412

Rao VD, Rao GKR (1983) Incidence of *Klebsiella* in foods and water. J Food Sci Technol 20 : 265-268

Scriven FM, Singh R (1986) Comparison of the microbial populations of retail beef and pork. Meat Sci 18 : 173-180

Simard RE, Auclair G (1981) Level of microbial contamination of some 'Canada approved' abattoirs and meat processing enterprises. J Canadian Inst. Food Sci Technol 14(2) : 128-134

Snedecor GW, Cochron WG (1968) Statistical Methods 1st edn Oxford and IBH Pub. pp. 258

Tibin IM, Melton CC (1990) Some microbiological assays of ground beef blended with hydrogenated soyabean oil. Meat Sci 28(3) : 245-249

Thatcher FS, Clark DS (1978) Microorganisms in Foods, 2nd edn. University of Toronto Press, Toronto-Buffalo-London

Tompkin RB (1983) Indicator organisms in meat and poultry products. Food Technol 37(6) : 107-110

Received 1 November 2000; revised 30 May 2001; accepted 13 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 82-84

Studies on Osmotic-Air Drying Characteristics of Papaya Cubes

S. KALEEMULLAH*, R. KAILAPPAN AND N. VARADHARAJU

Department of Agricultural Processing,
College of Agricultural Engineering,
Tamil Nadu Agricultural University, Coimbatore - 641 003, India

A study was conducted to investigate the effect of solute concentration (50, 60 and 70^0 Brix) and temperature (32, 50 and 60^{0}C) on osmotic dehydration of papaya. The dehydration rate of papaya cubes (6.58 kg water/kg dry matter) were reduced from 2.12 to 0.3, 2.38 to 0.31 and 2.92 to 0.54 kg water/h/kg dry matter during osmosis in first half an hour to fourth hour with the sugar syrup concentration of 50, 60 and 70^0 Brix, respectively at 32^{0}C temperatures. The dehydration rates of papaya cubes were reduced from 2.38 to 0.31, 3.16 to 0.29 and 4.17 to 0.44 kg water/h/kg dry matter during osmosis in first half an hour to fourth hour at 32, 50 and 60^{0}C temperatures, respectively at 60^0 Brix sugar syrup concentration. The overall drying rate of papaya by convective drying followed by osmotic dehydration was more (0.528 kg water/h/kg dry matter) than the one dried by convective method (0.318 kg water/h/kg dry matter). The osmotic (60^0 Brix, 60^{0}C) - air (60^{0}C) drying method saved 8 h in drying papaya from 6.58 to 0.24 kg water/kg dry matter as compared to the one dried by air drying (60^{0}C) method.

Keywords : Air-drying, Convective drying, Dehydration rate, Osmo-air drying, Osmotic dehydration, Papaya.

Papaya (*Carica papaya* L.) is an important fruit of tropical and sub-tropical regions of the world. The major producers of papaya in the world are Brazil, Thailand, Nigeria, Romania, India, Indonesia and Mexico. The world production of papaya was 5.02 million tonnes in 1997 (Vikas Singhal 1999) and it was reported that the loss was 40-100%. Papaya contains about 85% moisture (wb) and hence they are highly perishable in nature. The loss of papaya can be minimized to some extent, if the fruit is processed and stored. One way of processing the fruit is by osmotic-air drying and the osmotically dehydrated papaya can be easily accepted as a snack item compared to other processed fruit products with obvious economic advantages.

Osmotic dehydration is used for partial removal of water from materials such as fruits and vegetables (water containing cellular solids) by immersing the latter in a concentrated aqueous sugar or salt solution. The most commonly used osmotic agents are sucrose for fruits and sodium chloride for vegetables. The main advantages of osmotic dehydration include minimized heat damage, decreased bleaching of fruits (Ponting et al. 1966; Nanjundaswamy et al. 1978), with less damage to physical and chemical qualities of fruits (Adesina and Aina 1990). After initial dehydration by osmotic dehydration technique, the remaining water can be removed by air-drying upto a desired level. The objective of this study was to determine the drying parameters of papaya by osmotic-air drying technique.

Semi-ripened papaya fruits ('CO-2' variety) were obtained from Horticultural College and Research Institute Farm of Tamil Nadu Agricultural University, Coimbatore, India. The fresh fruits were washed in water to remove unwanted materials, peeled using a knife, cut into uniform cubes of 1.5 cm and dipped in 1% $CaCl_2$ solution for 10 min to increase firmness of papaya cubes.

Sugar syrups of 50, 60, 70^0 Brix were prepared with potassium metabisulphite (0.1%) and citric acid (0.1%) as preservatives. Initial experiment was carried out at room temperature (32^{0}C) to determine the effect of syrup (50, 60, 70^0 Brix) on dehydration of papaya. A solid to liquid mass ratio of 1:4 was maintained in all experiments. The papaya cubes were immersed in syrup and the beakers were kept in a thermostatic oven. Samples of papaya cubes were soaked in separate glass beakers containing sugar syrup for 0.5, 1, 2, 3, 4 h to know the quantity of water removed and the bone dry material present in it. After the set time interval, they were immediately removed and blotted using tissue paper. The cubes were weighed and their bone dry weights were determined by using a vacuum oven (AOAC 1995). The moisture content of osmosed papaya at each indicated time (0.5, 1, 2, 3, 4 h) was determined as follows.

$$\text{Moisture content of papaya after osmosis (kg water / kg d.m.)} = \frac{\text{Wt. of papaya after osmosis} - \text{Bone dry wt. of osmosed papaya}}{\text{Bone dry wt of osmosed papaya}}$$

The dehydration rates of osmosed papaya were calculated in between two successive timings i.e. 0 - 0.5, 0.5 - 1, 1 - 2, 2 - 3, 3 - 4 h, as it clearly gives an idea about dehydration rate in that particular individual period instead of overall dehydration rate at a particular time. The dehydration rate of papaya was calculated by using the following formula.

$$\text{Dehydration rate of papaya (kg water / h / kg d.m.)} = \frac{\text{Moisture content of papaya at '(t-1)' time} - \text{Moisture content of papaya at 't' time}}{\text{Dehydration time}}$$

The osmosed and raw papaya cubes were dried in a tray drier at 60 ± 2^{0}C and 30% relative humidity till the samples moisture content attained 0.25 kg water/kg dry matter. The reduction in weight was recorded at one-hour interval. The raw papaya dried in tray drier was taken as control. The experiments were done in triplicate.

The effect of osmotic concentration on dehydration of

*Corresponding Author

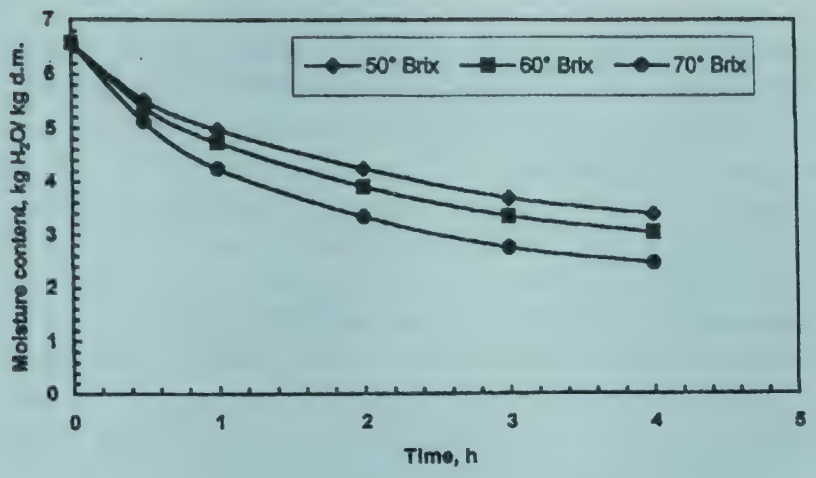

Fig. 1. Effect of osmotic syrup on the moisture content of papaya at 32°C

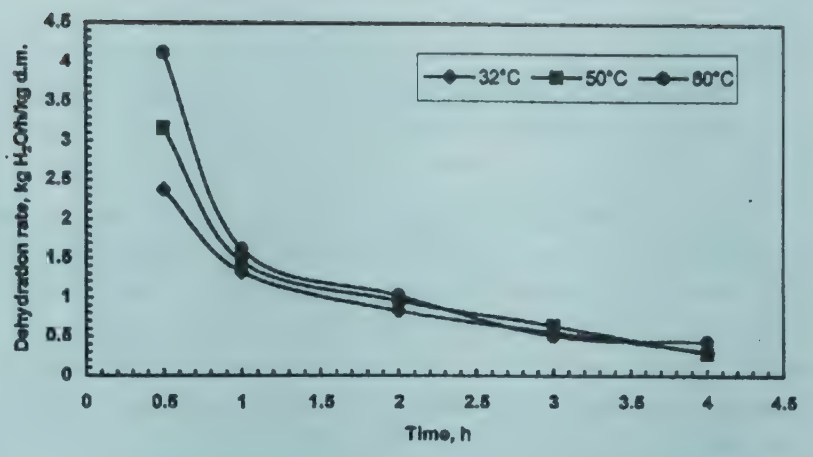

Fig. 4. Effect of temperature on the dehydration rate of papaya soaked in 60°B sugar syrup

Fig. 2. Effect of osmotic syrup on the dehydration rate of papaya at 32°C

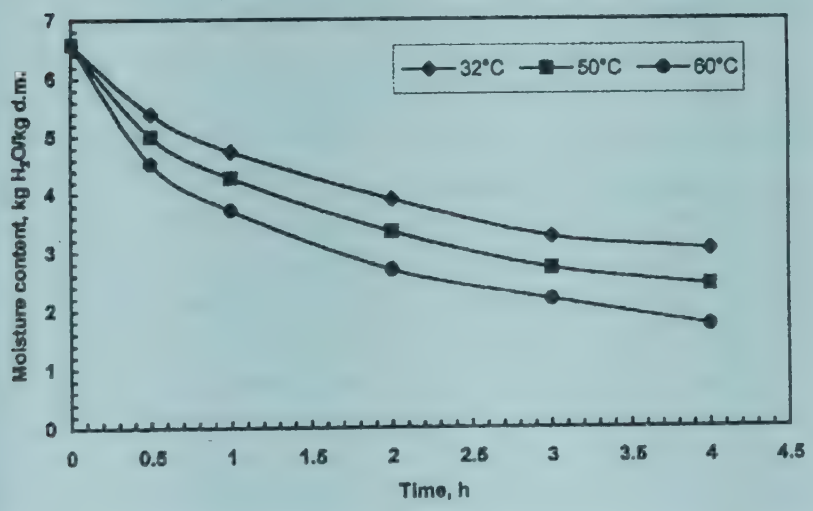

Fig. 3. Effect of temperature on the moisture content of papaya soaked in 60°B sugar syrup

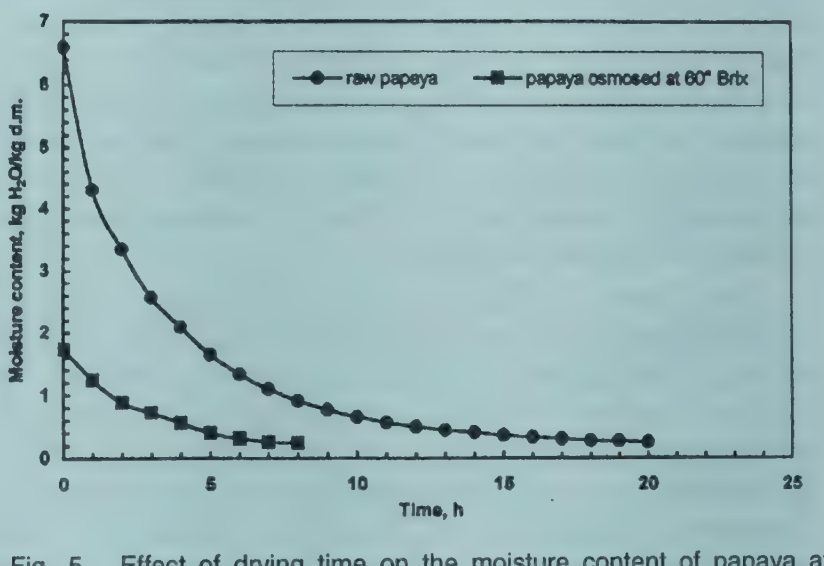

Fig. 5. Effect of drying time on the moisture content of papaya at 60°C

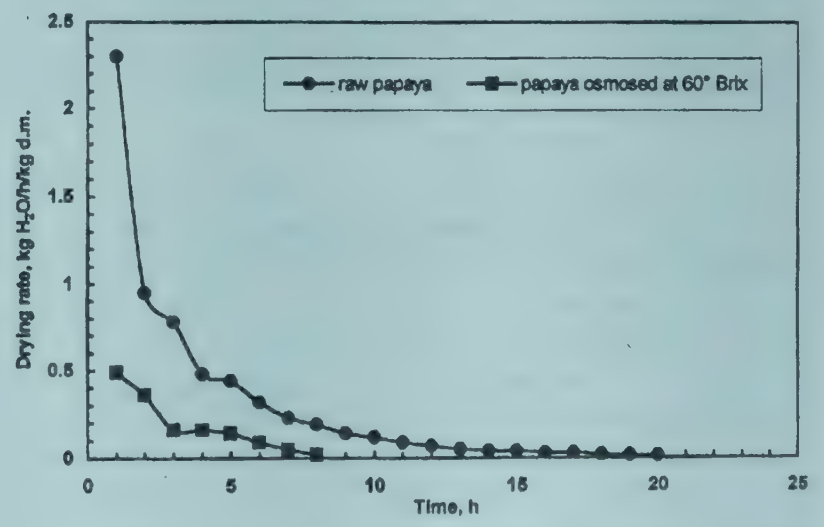

Fig. 6. Effect of time on the drying rate of papaya at 60°C air temperature

papaya at room temperature (32°C) is depicted in Fig. 1. It is clear that the moisture contents of papaya cubes were reduced substantially during osmosis at 50, 60 and 70° Brix sugar syrup respectively after 4 h. The dehydration of papaya increased with the increase in syrup concentration.

It is also clear that the dehydration rate was rapid initially and gradually decreased with time (Fig. 2). Similar result was reported in the case of osmotic dehydration of banana slices (Pokharkar et al. 1997). This is because the syrup gets diluted as a result of water movement from papaya to syrup. The overall osmotic dehydration rates in 4 h period were 0.8, 0.89 and 1.03 kg water/h/kg dry matter. It also showed that the dehydration rate was better in concentrated syrup due to the increased osmotic pressure in the sugar syrup at higher concentrations, which increased the driving force available for water transport. Even though, papaya gets dehydrated in

70^{0}Brix more, it was not considered for future experiment, as it needed heat to dissolve the sugar in the syrup. Further experiment was conducted at 60^0 Brix sugar syrup concentration, as the dehydration rate was more at 50^0 Brix.

Fig. 3 shows the effect of osmotic syrup temperature on dehydration of papaya at 60^0 Brix sugar syrup concentration. The moisture contents of papaya cubes. were considerably reduced from 6.58 to 3.03, 2.41 and 1.73 kg water/kg dry matter during osmosis at 32, 50 and 60^{0}C sugar syrup concentrations respectively after 4 h. The moisture removal from papaya increased with the increase of syrup temperature.

The dehydration rates of papaya cubes were reduced from 2.38 to 0.31, 3.16 to 0.29 and 4.12 to 0.44 kg water/h/kg dry matter during osmosis in the first half an hour to fourth hour at 32, 50 and 60^{0}C sugar syrup temperatures, respectively (Fig. 4). The overall osmotic dehydration rates in 4 h period were 0.89, 1.04 and 1.21 kg water/h/kg dry matter. This showed that the dehydration rate was more at higher syrup temperature. The reason was that the viscosity of hypertonic solution was lowered and the diffusion coefficient of water increased at higher temperature.

The effect of osmosis on convective drying time of papaya at 60^{0}C is presented in Fig. 5. It can be seen that the moisture content of osmosed papayas reduced from 1.73 to 0.24 kg water/kg dry matter within 8 h whereas in the case of raw papaya, the moisture content reduced from 6.6 to 0.251 kg water/kg dry matter within 20 h. The rate of dehydration of osmosed papaya and raw papaya in convective dryer reduced considerably from 0.49 to 0.02 and 2.3 to 0.014 kg water/h/kg dry matter within 8 and 20 h, respectively (Fig. 6).

The raw and osmosed papaya did not have any constant rate period and complete drying took place in falling rate period. The overall dehydration rate of osmosed papaya in convective drying was less (0.186 kg water/h/kg dry matter) than the raw one (0.318 kg water/h/kg dry matter) and correspondingly, the time taken by the osmosed papaya was also less. The reason was that the overall drying rate of papaya by convective drying followed by osmotic dehydration was more (0.528 kg water/h/kg dry matter) than the one dried by convective drying method (0.318 kg water/h/kg dry matter). The osmotic (60^{0}Brix, 60^{0}C) - air (60^{0}C) drying method saved 8 h in drying papaya from 6.58 to 0.24 kg water/kg dry matter than the one dried by air drying (60^{0}C) method only. Similar results have been reported by Amuthan et al. (1999) in the case of osmo-air drying of milky mushroom.

References

Adesina AA, Aina JO (1990) Preservation of African star apple fruits (*Chrysophyllum albidum*) by osmotic dehydration. Tropical Science 30(3) : 249-253

Amuthan G, Visvanathan R, Kailappan R, Sreenarayanan VV (1999) Studies on osmo-air drying of milky mushroom, *Calcocybe indica*. Mushroom Res 8(2) : 49-52

AOAC (1995) Official Methods of Analysis, 16th edn. Association of Official Analytical Chemists, Arlington, Virginia, USA

Nanjundaswamy AM, Radhakrishnaiah Setty G, Balachandran C, Saroja S, Murthy Reddy KBS (1978) Studies on dehydrated products from indigenous fruits. Indian Food Packer 1-2 : 91-98

Pokharkar SM, Suresh Prasad, Das H (1997) A model for osmotic concentration of banana slices. J Food Sci Technol 34 : 230-232

Ponting JD, Watters GG, Forrery RR, Jackson R, Stanley WL (1966) Osmotic dehydration of fruits. Food Technol 20(10) : 125-128

Vikas Singhal (1999) Papaya. In : Indian Agriculture, Indian Economic Data Research Centre, New Delhi, India. pp 169-170.

Received 21 December 2000; revised 31 May 2001; accepted 13 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 85-86

D-Value of Trypsin Inhibitor in Soybeans in Tomato Sauce

R.N.TRIPATHI[1*] AND NIRANKAR NATH[2]

Soybean Processing and Utilization Centre, Central Institute of Agricultural Engineering, Nabibagh, Berasia Road, Bhopal - 462 038, India.

[2]Department of Food Science and Technology, G.B. Pant University of Agriculture and Technology, Pantnagar - 263 145, India.

Thermal resistance of trypsin inhibitor (TI) in raw soybean flour blended in tomato sauce was determined at 82, 96 and 108^{0}C with holding time varying from 0 to 32.3 min. The TI activity reduced from 19.64 to 3.73, 2.75 and 2.51 mg/g at 82, 96 and 108^{0}C respectively. The D-Values for trypsin inhibitor were calculated as 47.6, 38.6 and 37.7 minutes at the above temperatures

Keywords : Soybeans, Tomato Sauce, Thermal resistance time, Trypsin inhibitor activity, D-value.

Soybean is a source of high quality protein but the raw beans contain undesirable trypsin inhibitor (TI) which has high thermal resistance (Savage et al. 1995). In addition, the beans have also other antinutritional factors like urease, hemagglutinins, phytic acid, allergens, saponins, goitrogens and flatulents (raffinose, stachyose and verescose) (Rackis 1974). TI is one among several other heat labile biologically active components present in soybeans and its inactivation is achieved by heat treatment (Liener 1962). Heat processing of soybeans apart from inactivating antinutritional components, is also known to improve their nutritional value through converting the native protein into denatured forms (Boonvisut and Whitaker 1976). Trypsin inhibitor is the most important among protease inhibitor (Klose et al. 1948) and its complete inactivation is not required to achieve optimum nutritive value (Rackis 1974). Kinetics of thermal inactivation of TI was found to be similar to the summation of two first order reactions with different heat stabilities (Johnson et al. 1980). It was probably due to the presence of a more heat sensitive Kunitz inhibitor and another Bowman-Birk inhibitor, which exhibited greater thermal resistance (Zuilichem et al. 1993).

The heat inactivation of TI is faster at higher pH than the lower pH, but the effect of pH become less pronounced in the higher temperature range (Kwok et al. 1993). Savage et al. (1995) found that TI was the least sensitive to heat and the optimal processing time based on acceptable inactivation time (AI), which was longest for TI, followed by urease and lipoxygenase. The purpose of this study was to determine the thermal resistance time for trypsin inhibitor of raw soy flour blend in tomato sauce (pH 4.30).

Soybean : Soybean variety, 'PK-472' was procured from Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar and was cleaned with grain cleaner.

Determination of thermal resistance of trypsin inhibitor: Soybean was ground to fine powder in a mini sample mill. Ten grams of ground powder were suspended in 100 ml tomato sauce (pH of sauce was 3.63 and pH of suspension was 4.30). Thermal resistance of TI was determine by TIT tube method (Ranganna 1994). Nine tubes were used for conducting experiment at each temperature. Suspension (2 ml) was pipetted using wide mouthed pipettes into each TIT tube and these tubes were sealed with rubber cork. The TIT tubes were made of Corning glass of 15 cm length, 1.48 cm outer diameter and 1.17 cm inner diameter. The selection of temperatures was based on the earlier work available on the inactivation of TI, so that the test tubes were heated in thermostatically controlled oil bath maintained at 82, 96 and 108^{0}C for varying periods upto to 40 min. After heating, the tubes were cooled immediately by putting them in ice water in a nice box and then, the samples were analyzed for residual trypsin inhibitor activity. TIA in the above samples were determined by the modified standard method of Hamerstrand et al (1981) and was calculated by using equation 1 and expressed in mg/g of sample.

$$\text{Trypsin inhibitor (mg/g)} = \frac{\text{Differential absorbance x dilution factor}}{0.019 \times 1000} \quad \text{-------- (1)}$$

Correction for come-up time : Time required for soyflour suspension in TIT tubes to attain the temperature of oil bath (i.e. Come-up time) was determined with the help of a 9.5 cm long needle-type plug-in copper constantan thermocouple (M/s Ecklund Inc. Cope, FL., USA). Temperature was recorded using a digital temperature indicator (Nutronics make, India). The correction to account for effectiveness of come- up time was calculated by using equations 2 and 3. Nath and Ranganna (1977) reported that only 42% of the come-up time of TIT tube contributed to lethality. Using the same value, correction for the come-up time was applied as follows

For heating time > come-up time;

Corrected heating time = Experimental heating time – 0.58 (come-up time) --------- (2)

For heating time < come-up time;

Corrected heating time = Experimental heating time x 0.42 ---------- (3)

Plotting of data : The residual TI activity vs corrected

*Corresponding Author

TABLE 1. RESIDUAL TRYPSIN INHIBITOR AND D-VALUE OF TI IN SOYFLOUR SUSPENDED IN TOMATO SAUCE (pH 4.30) AT DIFFERENT HEATING TEMPERATURES

Uncorrected heating time, min	Corrected heating time, min	Temperature, ^{0}C 82		96		108	
		TI, mg/g	D value, min	TI, mg/g	D value, min	TI, mg/g	D value, min
0	00.0	19.64		19.64		19.64	
5	02.0	16.71		16.33		14.01	
10	04.0	14.89		13.58		10.30	
15	07.3	12.71		12.02		07.82	
			47.6		38.6		37.7
20	12.3	10.90		08.02		05.41	
25	17.3	08.53		05.56		03.98	
30	22.3	06.70		04.22		03.31	
35	27.3	05.07		03.97		02.84	
40	32.3	03.73		02.75		02.51	

Degree of freedom = 7 r = 0.96 - 0.99

pH of tomato sauce was 3.63

heating time curve was plotted on semilog paper with TI activity on the log axis. Survivor curves were drawn by applying analysis of least squares (Perneski et al 1973). D-values of TI in the temperature range of 82 to 108^{0}C were calculated as negative inverse of the slope of the best fit line. Thermal resistance curve was obtained by plotting D-values against corresponding temperatures on a semilog paper with D-values on the log axis by applying analysis of least squares.

The residual TIA of soyflour suspended in tomato sauce (pH 4.30) is reported in Table 1. The TI activity reduced from 19.64 to 3.73, 2.75 and 2.51 mg/g of sample on heating at 82, 96 and 108^{0}C respectively for 32.3 min., and the extents of reductions were 81%, 86% and 87% respectively. The survivor curves of TI in raw soyflour suspended in tomato sauce (pH 4.30) were straight lines (R^2 ranged from 0.93 to 0.99).

The D-values for TI of soyflour suspended in tomato sauce (pH 4.30) were 47.6. 38.6 and 37.7 minutes at 82, 96 and 108^{0}C respectively (Table 1). The D-value decreased with an increase in the temperature of the heating medium.

References

Boonvisut S, Whitaker JR (1976) Effect of heat amylase and disulfide bond cleavage on the in-vitro digestibility of soybean proteins. J Agric Food Chem 24 : 1130.

Hamerstrand GE, Black LT, Glover JD (1981) TI in soy products: Modification of the standard analytical procedure. Cereal Chem. 58 : 42-45

Johnson LA, Deyoe CW, Hoover WJ, Schwenke JR (1980) Inactivation of trypsin inhibitor in aqueous soybean extracts by direct steam infusion. Cereal Chem 57 : 376-379.

Klose AA, Hill B, Fevold HL (1948) Food value of soybeans as related to processing. Food Technol 2 : 201-206

Kwok KC, Qin WH, Tsang JC (1993) Heat inactivation of trypsin inhibitors in soymilk at ultrahigh temperatures. J Food Sci 58 : 859-862.

Liener IE (1962) Toxic factor in edible legumes and their elimination. Am J Clin Nutr 11 : 281-298.

Nath N, Ranganna S (1977) Evaluation of thermal process for acidified canned muskmelon (*Cucumis melo L.*). J Food Sci 42 : 1306-1310.

Perneski BW, McDonald LL, Milliken GA (1973) Use of spline functions in analyzing submarine exercise data. Naval Analysis Programs of Naval Research, Arlington, VA.

Rackis JJ (1974) Biological and physiological factors in soybeans. J Am oil Chem Soc 51 161A-174A.

Ranganna S (1994) Handbook of Analysis and Quality Control for Fruits and Vegetables Products. Tata McGraw Hill Publishing Co. Ltd. New Delhi.

Savage WD, Wei LS, Sutherland JW, Schmidt SJ (1995) Biological active components inactivation and protein insolubilization during heat processing of soybeans. J Food Sci 60 : 164-180

Zuilichem DT V, Adriaansen PP, Hok A, Hin R, Stolp W, Poel AFB Van-der (1993). Kinetic behavior of soybeans during heat treatment. Voedingsmiddelentechnologie 26 : 19-22

Received 27 December 1999; revised 7 June 2001 accepted 13 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 87-90

Optimization of Processing Conditions for Cottage Scale Production of *Hurum*

CHARU LATA MAHANTA* AND RITU GEU GOSWAMI

Department of Foods and Nutrition,
College of Home Science,
Assam Agricultural University,
Jorhat - 785 013, India.

A cottage industry level process for *hurum,* a waxy rice product of Assam was developed in a flaked rice mill. Optimum conditions of soaking, parboiling, flaking and expansion of waxy rice into *hurum* were determined. Addition of fat increased expansion. Thinner flakes gave higher expansion ratio. The process involved overnight soaking of paddy in just-boiled water, parboiling by sand roasting for 1 min at 200^{0}C in sand, flaking of the roasted paddy, application of fat to the flaked rice followed by expansion in sand at 200^{0}C for 14 sec. Moisture level in the flaked rice was critical to expansion.

Keywords : Waxy rice, Expanded rice, Rice product, *Hurum,* Cottage Scale process for *hurum.*

Expanded rice is a popular rice product in the Indian sub continent (Bhattacharya and Ali 1985). Both the non-waxy rices and waxy rices are processed into expanded rice. In Assam, the north-eastern State of India, expanded non-waxy rice product is called *muri,* while that from waxy rice is called *hurum.* The processing steps for making *hurum* differ from those of *muri.* Standardized laboratory scale and cottage scale processes for *muri* exist (Chinnaswamy and Bhattacharya, 1983 b). *Hurum*-making process, however, is still very traditional. A tentative laboratory process for *hurum* has been developed (Mishra et al. 2000). However, its cottage scale process has not been developed so far.

The essential steps in the traditional process for making *hurum* as identified in the laboratory (Mishra et al. 2000) are - (i) parboiling of soaked paddy by the conventional method, (ii) flaking of high-moisture paddy, (iii) application of fat to the moist flaked rice, and (iv) its expansion in hot sand. Based upon the essential steps identified for making *hurum,* an attempt was made to identify the optimum process variables in the processing of waxy rice into *hurum* in a flaked rice mill and the results are reported in this communication.

Mill : A flaked rice mill in Pokamara, Jorhat, in Assam was selected. In this mill, flaked rice is prepared from dry-heat parboiled rice. The furnace is husk-fired. The iron pan used for parboiling is kept in a slanted position at a level such that stirring of paddy-sand mixture is done in a standing position. Flaking is done in a single edge-runner run on diesel.

Paddy : 'Bhogali bora' a waxy rice variety released by the Regional Agricultural Research Station, Assam Agricultural University, Titabor was used for the study.

Parboiling : Two soaking conditions were studied. In the first method, paddy was brought to boil in water and left overnight (16-18 h) for hydration. In the second method, paddy was added to boiling water, stirred well and kept overnight for hydration. For overnight hydration, the vessel was covered well with jute sacks. Paddy to water ratio used was 1:1.5. The water was drained off the next morning.

Soaked paddy was parboiled at three sand temperatures of 180^{0}C, 200^{0}C and 220^{0}C for 1 min. The sand available from the river bed locally was very fine, with a major quantity passing through 72 mesh and negligible amount was of +30 size. The sand used for parboiling was of -30+52 mesh, as initial studies with -72 mesh size sand showed that the sand adhered to the expanded grains. The temperature of sand was recorded by a dial thermometer. Parboiling of paddy was done at three paddy to sand ratios of 1:2, 1:3 and 1:4. During parboiling, the paddy-sand mixture was vigorously stirred. The parboiled paddy and sand mixture was immediately lifted on a wire mesh sieve to sieve away the sand. The parboiled paddy was spread on jute sacks in shade to cool.

Flaking : The cooled paddy was then flaked in a single edge-runner for about 1.30 to 2 min. As the flaking was to be done when the parboiled paddy was still moist (as explained later) the husk could not be fully separated from the flaked rice. Remaining husk was removed by winnowing. Care was taken to prevent the moist-flaked rice from losing moisture.

Application of fat : The flaked rice was thoroughly rubbed with fat using both palms. The effect of three types of fat was studied. The fats used were hydrogenated fat, ghee and refined vegetable oil. Fat was added in three levels, viz., 750:7.5, 750:15, and 750:22.5 of flaked rice to fat ratio (by weight).

Heat expansion : Flaked rice (150 g) was either immediately expanded or rested for 60 min in a covered vessel before expansion. Expansion was done in the same iron pan used for parboiling. The flaked rice was expanded in sand (-30+52 mesh) heated to various temperatures between 200^{0}C and 250^{0}C for 8-14 sec. The expansion was carried out in kernel to sand ratios of 1:10, 1:15 and 1:20. The rice-sand mixture was vigorously stirred and lifted on to a wire-mesh sieve to remove the sand. Expansion ratio was measured in triplicate for each variable.

**Corresponding Author*

Other methods : Amylose content of 'Bhogali bora' was determined according to the method of Sowbhagya and Bhattacharya (1979). The length, breadth and thickness of 20 grains were measured by vernier calipers and average measurements are expressed in mm. The expansion ratio was determined by measuring the bulk volumes of 150 g moist flaked rice and the resulting expanded rice in 250 ml and 1000 ml graduated measuring cylinders, respectively, after tapping the cylinder for 15 times on a folded Turkish cloth kept on a wooden plank.

Moisture content of the paddy at various processing steps and flaked rice was determined in triplicate using a moisture meter (Model, SS-5, Japan). The moisture meter was calibrated against moisture estimated by the oven method (AACC 1962). The thickness of 10 flaked rice was measured with the help of vernier calipers. The mean values are reported.

Characteristics of 'Bhogali bora' : Milled 'Bhogali bora' had an amylose content of 4.5% (d.b.) the mean grain length, breadth and thickness were 7.4 mm, 3.0 mm, and 2.5 mm, respectively. The length/breadth ratio was 2.5.

Optimum parboiling conditions : High moisture of the soaked paddy is essential for *hurum* processing as reported in the traditional process (Mishra et al. 2000). In the traditional process, waxy paddy is soaked at RT for 3-5 days for complete hydration wherein paddy attains a moisture level of 35% (w.b.). Of the two soaking methods studied, the first method of bringing paddy (3-4 kg lots) to boil in water raised the paddy moisture level to about 40% (w.b.). The high moisture level after parboiling caused problems of lumping during the flaking process and hence was abandoned. The second method of adding paddy to boiling water raised the moisture level to 35% (w.b.), which could be satisfactorily parboiled and flaked without lump formation. Hence, the second method of soaking paddy was followed for *hurum* processing.

Out of the three paddy to sand ratios studied for dry-heat parboiling, paddy to sand ratios of 1:2 and 1:3 were found to bring down the moisture level within the desirable level (explained later) for flaking and were selected for further studies. Paddy to sand ratio of 1:4 caused excessive drying of the kernels that disintegrated into pieces during the flaking process.

Flaked rice directly expanded without fat application gave poor expansion. When flaked rice that was rubbed with fat was immediately expanded, too much of sand remained sticking to the expanded grain and the expansion was not optimum. A resting time of 60 min was, therefore, given to fat-coated flaked rice for further studies.

Moisture levels and flaked rice thickness of variously parboiled paddy : The paddy-sand mixture temperature at the end of parboiling fell by 60 - 80^{0}C depending on the initial sand temperature. The immediate moisture levels of parboiled paddy and flaked rice decreased with increasing initial sand temperature used for parboiling (Table 1). Similarly, the thickness of flaked rice was also high for parboiled paddy with high moisture. Further flaking of high moisture parboiled rice caused lumping of the sticky grains.

Effect of paddy to sand ratio : Both 1:2 and 1:3 paddy to sand ratios with initial soaked paddy weight of 2 kg were found to give similar expansion ratios (Table 2). However, 1:3 ratio was observed to be very laborious for stirring while dry-heat parboiling compared to 1:2 ratio.

Effect of sand temperature during parboiling : Sand-roasting temperature of 200^{0}C gave the highest expansion ratio followed by temperatures of 180^{0}C and 220^{0}C, in that order (Table 2). Sand temperatures above 220^{0}C were not tried as higher temperatures would dry up the parboiled grain that would not yield moist flaked rice essential for hurum making.

Heat expansion conditions : The heat expansion conditions followed for making expanded rice from non waxy rice in a cottage industry reported by Chinnaswamy and Bhattacharya (1983 b) were roasting with sand of parboiled rice of 11% moisture (w.b.) in 1:10 paddy to sand ratio at 240^{0}C - 250^{0}C for 11-13 sec. These authors also observed that sand temperature, sand quantity and rice quantity were interrelated and a different set of optimum conditions could be obtained with different sand temperatures and sand and rice quantities.

In the present study, flaked rice was expanded at various sand temperatures for 11 and 14 sec (Table 2). While hurum remained somewhat soft when expanded for 11 sec at sand temperatures for 200^{0}C and 220^{0}C, that expanded at 240^{0}C and 250^{0}C gave slightly coloured product. The expansion ratio was also not good. However, expansion for 14 sec at 200^{0}C gave better ratio of 7.4 compared to 5.7 at 11 sec and the product obtained was crisp.

In comparison, 'Asm 44' (a waxy rice of Assam) was reported to give an expansion ratio of 4.3 when processed by the muri-making process (Chinnaswamy and Bhattacharya,

TABLE 1. MOISTURE LEVELS DURING PROCESSING OF SOAKED PADDY AND THICKNESS OF FLAKED RICE[a]

Soaked paddy moisture, % w.b.	Parboiling conditions: Sand temp, ^{0}C	Parboiling conditions: Paddy sand mixture final temp, ^{0}C	Parboiled paddy moisture, % w.b.	Flaked rice: Moisture, % w.b.	Flaked rice: Thickness, mm
35.0	180	118	19.0	22.7	1.33
	200	138	17.0	20.3	1.24
	220	144	15.9	18.2	1.19

[a] Paddy : sand ratio 1:2, Heating time 60 sec.

TABLE 2. EFFECT OF PROCESSING CONDITIONS ON THE EXPANSION RATIO OF WAXY RICE

Variable studied	Parboiling			Heat expansion			Fat expansion		Expansion ratio
	Paddy : sand ratio	Sand temp, ^{0}C	Flaked rice-sand ratio	Sand temp, ^{0}C	Time sec.	Fat type	Flaked rice : fat ratio	Resting time, sec.	
A. Thick flakes (1.19-1.33 mm thickness)									
Paddy:sand ratio	1:2	200	1:20	200	14	H[a]	750:15	60	8.9
	1:3								
Parboiling sand temp.	1:2	180	1:20	200	14	H	750:15	60	8.9 8.5
		200							8.9
		220							7.4
Flaked rice: sand ratio	1:3	180	1:10	200	14	H	750:15	60	7.9
			1:15						8.3
			1:20						8.5
		200							8.9
		220							7.4
Sand expansion temp.	1:2	220	1:20	200	11	H	750:15	60	5.7
					14				7.4
				220	11				6.1
				240					6.6
				250					7.0
	1:3	220	1:20	200	11	H	750:15	60	6.0
					14				7.3
				220	11				6.3
				240					6.2
				250					6.0
Fat quantity	1:2	180	1:20	200	14	0	0	0	4.5
						H	750:15	0	5.8
								60	8.6
							750:7.5		7.7
							750:22.5		9.1
Fat type	1:2	180	1:20	200	14	H	750:15	60	8.5
						G[b]			8.6
						R[c]			7.0
		200				H			8.9
						H			9.6
						R			7.4
B. Thin flakes (0.90 mm thickness)									
Flaked rice thickness	1:3	180	1:20	200	14	H	750:15	60	9.9
						G			9.9
						R			9.3

[a]Hydrogenated fat, [b]Ghee, [c]Refined oil

1983 a). The moisture content of the waxy rice before expansion was 11% (w.b.) and expansion was done at 250^{0}C sand temperature. The authors further observed that waxy rices gave the lowest expansion among the other rices varying in amylose content. On the other hand, Antonio and Juliano (1973) reported that the extent of expansion was inversely related to the amylose content, being maximum in waxy rice. However, the expansion ratio reported by Antonio and Juliano (1973) was much lower than that achieved in the present study.

Effect of quantity and type of fat : In the traditional hurum-making process (Mishra et al. 2000), fat is rubbed on to flaked rice before it is heat-expanded to increase the expansion ratio as against salt solution that is applied to milled non waxy parboiled rice before heat expansion (Chinnaswamy and Bhattacharya 1983 b).

Flaked rice expanded without rubbing of fat gave poor expansion ratio of 4.5 (Table 2). When hydrogenated fat was applied, expansion ratio increased considerably. For a flaked rice to fat ratio of 750:15, the expansion ratio was 5.8, when the rice was expanded immediately after fat application. However, with a resting time of 60 min. the same flaked rice gave an expansion ratio of 8.6. The quantity of fat applied appeared to influence expansion ratio. The ratios increased with increase in fat. These were 7.7, 8.6 and 9.1 for 7.5 g, 15.0 g and 22.5 g hydrogenated fat, respectively, that was applied to 750 g of flaked rice followed by a resting time of 60 min. However, with increase in fat, the sand used for

expansion also became very oily.

Next, the effect of three types of fat on expansion ratio was studied. Ghee is traditionally used to make hurum. Hydrogenated fat and refined oil being cheaper than ghee were the other two fats used. It was observed that while ghee caused the highest expansion, refined oil caused the lowest (Table 2). The higher expansion due to ghee, contrary to Mishra et al's (2000) view, could probably be due to the effect of resting time of 60 min.

Effect of thickness of flaked rice : Whether thickness of flaked rice influenced expansion ratio or not was determined. As further thinning of flaked rice of 1.33 mm thickness obtained from parboiled paddy roasted at 180^{0}C caused lumping of the grains, the flaked rice was, therefore, mixed with rice bran powder and then further flaked. This helped to achieve thinner flakes (0.90 mm thickness). The thin flakes after fat application and heat expansion yielded still high expansion ratio (Table 2). The ratio was 9.9 with hydrogenated fat or ghee and 9.3 with refined oil.

Optimum conditions for making hurum *:* The optimum conditions can be surmised from these findings. Waxy paddy should be soaked overnight by adding to just-boiled water removed from fire. The drained paddy should be roasted in sand (paddy to sand ratio of 1:2) for 1 min at 200^{0}C, cooled for a few min, flaked, rubbed with fat (750:15 flaked rice to hydrogenated fat ratio) and heat expanded at 200^{0}C for 14 sec in 150 g lot with 20 times its weight of sand. *Hurum* thus obtained is crisp and mostly translucent in appearance with some opaque white parts (Plate 1). It is not wholly opaque as muri.

Critical factors for optimum expansion : Besides complete gelatinisation of starch during parboiling, the critical factors appear to be the moisture level of the parboiled rice preparatory to expansion (in the present study this ranged from 18%-23%, w.b.) and the flaking of the parboiled rice. Probably, high moisture content is essential to expand amylopectin-rich starch and flaking probably helps in the easy escape of moisture by increasing the surface area of the rice grain. Fat application possibly increases the heat transferred to the grain during expansion. These aspects need to be further studied.

Further work on hurum *:* Since processing of *hurum* uses both fat and high temperature, the product has a short shelf life. This is not a problem when processing is done by the traditional process for home consumption. Further studies on

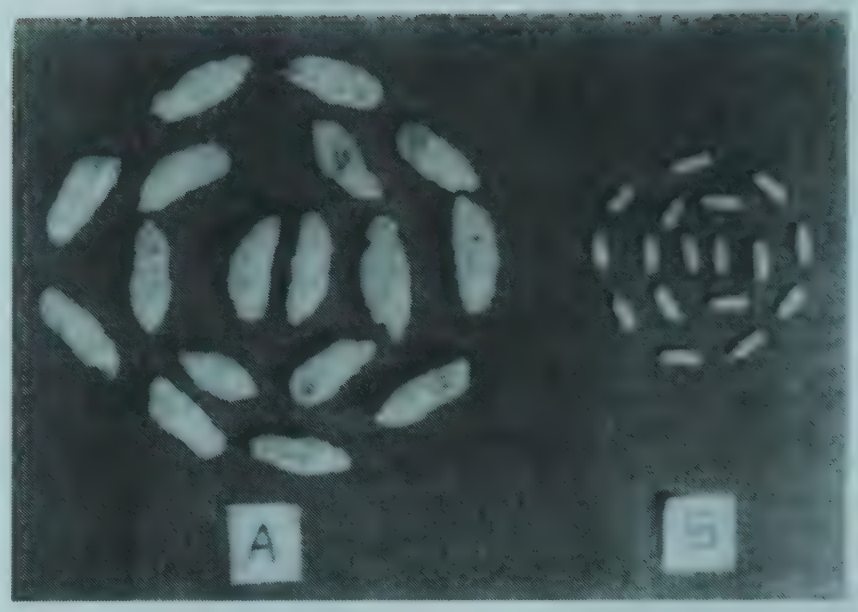

Fig. 1. (a) *Hurum* and (b) raw rice (*Bhogali bora*)

shelf life and prevention of off odour is presently underway. The economics of commercial production of *hurum* as a cottage industry is being worked out.

This work was supported by research grant from ICAR under the *Ad hoc* research project on "Physico-chemical Basis for the Puffing Quality of the Waxy Rices of Assam".

References

AACC (1962) Approved Methods of Analysis. 8th revised edn. American Association of Cereal Chemists. St Paul. MN. USA

Antonio AA, Juliano BO (1974) Physicochemical properties of glutinous rices in relation to pinipig quality. Phillip Agric. 58 : 17-23

Bhattacharya KR, Ali SZ (1985) Changes during parboiling and properties of parboiled rice. In : Adv. Cereal Chem, St. Paul, MN, USA. Pp 105-167

Chinnaswamy R, Bhattacharya KR (1983 a) Studies on expanded rice. Physicochemical basis of varietal differences. J. Food Sci 48 : 1600-1603

Chinnaswamy R, Bhattacharya KR (1983 b) Studies on expanded rice. Optimum processing conditions. J. Food Sci 48 : 1604-1608

Mishra A, Goswami RG, Mahanta CL (2000) Identifying the essential steps and process variables to make *hurum*. J. Food Sci Technol 37 : 455-458

Sowbhagya CM, Bhattacharya KR (1979) Simplified determination of amylose in milled rice. Staerke 31 : 159-163

Received 17 April 2000; revised 7 June 2001; accepted 14 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 91-93

Effect of Pasteurization Methods on the Quality Characteristics of Yolk and Sponge Cakes

A. BHARTI, P.C. PANDA AND J. SAHOO*

Department of Animal Products Technology,
CCS Haryana Agricultural University, Hisar - 125 004, India.

The effects of pasteurization methods and addition of sugar on physico-chemical properties of yolk and certain quality characteristics of sponge cakes were studied. The results indicated that solid content was significantly higher and emulsion stability was lower in sugared yolks as compared to plain yolks irrespective of pasteurization methods. The cake volume was significantly ($p<0.05$) lower in both conventional and ultra-pasteurization methods as compared to unpasteurized sample. Pasteurization method could not significantly improve the overall acceptability of the product.

Keywords : Pasteurization techniques, Egg yolk quality, Sponge cake, Cake volume, Organoleptic quality.

It is estimated that around 3 million metric tonnes of liquid egg products are produced annually in the world, which forms 9% of total table egg output. Since liquid egg before being diverted for preparation of various products should be pasteurized and conventional methods of pasteurization are being considered insufficient to eliminate some of the pathogens recently identified from eggs, the emphasis has shifted to ultra pasteurization, hyper pasteurization and other novel techniques (Panda 1994). Hence, the present study was undertaken to assess the effect of ultra pasteurization of the quality of yolk and sponge cakes.

Collection of sample : Fresh eggs (less than two days old) from White Leghorn hens of same age and reared under similar conditions were collected in lots of 50. Fortyfive eggs were further selected and divided into 3 groups of 15 each. Eggs were then dipped into ethanol and air dried. Each group of eggs was broken aseptically and the yolk was separated. Yolk content of group I was kept as control i.e. unpasteurized, while that of group II and III were subjected to conventional pasteurization (61.1^{0}C/3.5 min) and ultra pasteurization (68^{0}C/ 95 sec), respectively. Total liquid yolk obtained from each group was divided into two halves. One half was plain and the other half was sugared with 6.5% fructose, thus making a total of 6 experimental groups. These were then frozen and stored for 20 days at -10^{0}C. On the day of analysis, the yolk samples were removed, thawed at room temperature (25^{0}C) for 50 min and examined for different physico-chemical properties such as % total solids and pH by using a digital pH meter directly into the yolk (AOAC 1995).

Emulsion stability : Emulsion stability of yolk was determined by the method of Jaax and Travnicek (1968) with slight modification. Emulsion was prepared with 8.5 g yolk, 11 g refined oil and 460 ml triple distilled water using kitchen aid mixer for a period of 90 sec at high speed. The emulsions were allowed to set at room temperature for 3 h in 100 ml measuring cylinders. The quantity of oil separated at the end of 3 h were recorded and expressed as emulsion stability.

*Corresponding Author

Preparation of sponge cake : The recipe used for the preparation of sponge cake consisted of defrosted egg yolk 60 g, water 41 ml, common salt 0.375 g, granulated sugar 62.5 g, refined wheat flour 42 g and baking powder 1.35 g.

Sugar (25 g) was shifted three times with 24 g of refined wheat flour. Baking powder was added in the remaining flour and sieved thrice. Yolk-water-salt mixture was prepared by adding boiling water and salt in yolk beaten for 5 min at medium speed of a kitchen aid mixer. Sugar (37.5 g) was added to the yolk-water-salt mixture at the rate of one tablespoon at a time and the mixer was allowed to beat for another 1.5 min at the same speed. The sifted flour sugar mixture was added to it at the rate of one tablespoon at a time and was beaten for 1 min at slow speed. At last the shifted flour-baking powder mixture was added and beaten for 2 min to get the batter.

The batter (135 g) was poured into a 19 cm x 8.9 cm x 6.4 cm loaf pan lined with wax paper and baked at 177^{0}C for 35 min. The cakes were cooled for 1 h and cake volumes were recorded by seed displacement method (Jaax and Travnicek 1968). A panel of 6 judges evaluated the organoleptic quality of the sponge cake in respect of shape, colour, texture, flavour and overall acceptability following a 7 point Hedonic scale (1 = very poor and 7 = excellent).

Yolk quality : Effect of pasteurization on physico-chemical property of yolk (Table 1) showed that the total % solids of the conventional as well as ultrapasteurized plain yolk were significantly ($P < 0.05$) lower as compared to the sugared yolk. Further, it was observed that the total % solids in ultrapasteurised sugared yolk was significantly lower as compared to that in the conventional method and unpasteurized sample. The observation were consistent with the findings of Martinez et al. (1995) who reported that the solid contents of yolk were mainly affected by the time rather than temperature used in the pasteurization technique. The emulsion stability was significantly lower in sugared yolk than in plain yolk whether pasteurized or not. This was in conformity with earlier finding of Jaax and Travnicek (1968). Further, it was observed that emulsion stability of sugared yolk was significantly higher in ultrapasteurized condition as compared to conventional

TABLE 1. EFFECT OF PASTEURIZATION METHODS ON PHYSICO-CHEMICAL PROPERTIES OF YOLK (MEAN ± SE)

Type of yolk	Total solids, %	Emulsion stability, % of oil separation	pH
Unpasteurized plain	$45.98^{b} \pm 0.45$	$7.91^{a} \pm 0.06$	$7.00^{a} \pm 0.03$
Unpasteurized sugared	$48.54^{d} \pm 0.17$	$18.97^{c} \pm 0.09$	$6.96^{a} \pm 0.02$
Conventionally pasteurized plain	$43.12^{a} \pm 0.16$	$9.18^{b} \pm 0.18$	$6.94^{a} \pm 0.01$
Conventionally pasteurized sugared	$48.44^{d} \pm 0.14$	$19.84^{d} \pm 0.11$	$6.98^{a} \pm 0.01$
Ultrapasteurized plain	$42.90^{a} \pm 0.40$	$8.87^{b} \pm 0.07$	$7.54^{b} \pm 0.06$
Ultrapasteurized sugared	$46.82^{c} \pm 0.11$	$18.90^{c} \pm 0.18$	$7.48^{b} \pm 0.03$

Figures with different superscripts in the same column differ significantly at $P < 0.05$.

TABLE 2. EFFECT OF PASTEURIZATION METHODS OF YOLK ON CAKE VOLUME AND ORGANOLEPTIC CHARACTERISTICS OF SPONGE CAKES (MEAN ± SE)

Type of yolk	Cake volume, ml	Shape	Colour	Texture	Flavour	Overall acceptability
Unpasteurized plain	$61.46^{b} \pm 0.82$	$5.33^{c} \pm 0.10$	$3.58^{a} \pm 0.08$	$4.91^{a} \pm 0.15$	$5.33^{b} \pm 0.10$	$5.25^{b} \pm 0.11$
Unpasteurized sugared	$60.42^{b} \pm 0.80$	$5.16^{b} \pm 0.10$	$4.16^{c} \pm 0.10$	$4.83^{a} \pm 0.10$	$5.00^{a} \pm 0.12$	$4.83^{a} \pm 0.10$
Conventionally pasteurized plain	$55.88^{a} \pm 0.93$	$5.50^{c} \pm 0.12$	$3.50^{a} \pm 0.12$	$4.83^{a} \pm 0.10$	$4.91^{a} \pm 0.15$	$5.00^{a} \pm 0.12$
Conventionally pasteurized sugared	$55.36^{a} \pm 0.94$	$4.50^{a} \pm 0.12$	$4.33^{c} \pm 0.10$	$4.58^{a} \pm 0.08$	$4.75^{a} \pm 0.11$	$4.83^{a} \pm 0.10$
Ultrapasteurized plain	$56.28^{a} \pm 3.54$	$5.66^{c} \pm 0.10$	$4.00^{b} \pm 0.18$	$4.75^{a} \pm 0.11$	$5.08^{a} \pm 0.15$	$5.33^{b} \pm 0.10$
Ultrapasteurized sugared	$53.82^{a} \pm 0.89$	$4.50^{a} \pm 0.12$	$4.41^{c} \pm 0.08$	$4.66^{a} \pm 0.10$	$4.75^{a} \pm 0.11$	$4.83^{a} \pm 0.10$

Figures with different superscripts in the same column differ significantly ($P < 0.05$).

pasteurization, whereas, no statistical difference was observed in the plain yolk treated with above pasteurization techniques. This could be explained based on the observation made by Varadarajulu and Cunningham (1972), who observed that the reduction in solid content in ultrapasteurized sample due to less exposure time might be playing a role in the differences in emulsion stability of ultra and conventionally pasteurized samples.

Results pertaining to effect of these pasteurization techniques on pH of yolk (Table 1) indicated that the pH of both ultrapasteurized plain and sugared yolk was significantly higher ($P < 0.05$) than those of conventional pasteurized or unpasteurized. This was consistent with earlier findings of Martinez et al. (1995).

Sponge cake quality : The cake volume in case of unpasteurized plain and sugared yolk (Table 2) was significantly higher than that of the pasteurized yolks. No significant difference in the cake volume between the conventional and ultrapasteurized groups was observed. This was consistent with the earlier findings of Jaax and Travnicek (1968). Stadelman and Cotteril (1977) indicated that sugar depressed the foaming capacity. Perhaps this was the reason why in case of frozen sugared yolk, the volume of sponge cake was consistently low irrespective of pasteurization techniques employed. The organoleptic scores (Table 2) for cake shape in plain yolk whether pasteurized or not, was significantly ($P < 0.05$) higher as compared to that in sugared yolk. On the other hand, unpasteurized sugared yolk showed a significantly ($P < 0.05$) higher scores for cake shape as compared to those of pasteurized sugared yolk. Colour scores were significantly ($P < 0.05$) higher in sugared frozen yolk when compared to plain yolk. The texture scores of the sponge cake made of sugared or plain yolk whether pasteurized or not, did not differ significantly. Flavour scores were significantly ($P < 0.05$) higher in unpasteurized plain group than in other experimental group.

Results of overall acceptability scores were significantly higher for the cakes prepared from unpasteurized as well as ultrapasteurized plain yolks, compared with other experimental groups. Sugared yolk either pasteurized or unpasteurized were not superior to the plain yolk for preparation of the sponge cakes except colour.

References

AOAC (1995) Official Methods of Analysis. 21st edn, Association of Official Analytical Chemists, Washington DC

Jaax S, Travnicek D (1968) The effect of pasteurization, selected additives and freezing rate on the gelation of frozen defrosted egg yolk. Poult Sci 47 : 1013-1022

Martinez RM, Dawson PL, Ball HR Jr, Swartzel KR, Winn SE, Giesbercht FG (1995) The effects of ultrapasteurization with and without homogenization on the chemical, physical and functional properties of aseptically packaged liquid whole egg. Poult Sci 74 : 742-752

Panda PC (1994) Poultry processing in India - Current status and future vision. In : Proceedings of the National Symposium on Meat and Milk Industry : Trends and Development Strategies. pp. 61-66

Stadelman WJ, Cotterill OJ (1977) Egg Science and Technology. 2nd ed. AVI Publishing Company, inc, Westport, Connecticut, USA

Varadarajulu P, Cunningham FE (1972) A study of selected characteristics of hens egg yolk. 2. Influence of processing procedures, pasteurization and yolk fractionation. Poult Sci 51 : 941-945

Received 25 May 2000; revised 7 June 2001; accepted 14 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 94-95

Physico-chemical Changes During Ripening of Guava Fruit *(Psidium guajava L.)*

RAVI AGRAWAL*, P. PARIHAR, B.L. MANDHYAN AND D.K. JAIN

Department of Post Harvest Process and Food Engineering,
J.N. Krishi Vishwa Vidyalaya, Jabalpur - 482 004, India.

Physical and biochemical changes were studied in guava over the maturity period. The maturity was defined in terms of changes of colour from green to yellow. The results revealed that the weight of guava increased from 36.82 g/cm size at 100% green to 50.24 g/cm size at 100% yellow. The true density decreased from 1.03g/cc to 0.91 g/cc. The total soluble solids increased from 10.5% to 12.75%, and total soluble sugars varied from 4.81g/100 g to 7.32 g/100 g. It was further observed that ascorbic acid content increased from 118.53 g/100 g to 199.26 g/100 g, while the percentage acidity decreased from 0.72 to 0.55%.

Keywords : Guava flesh, Seed ratio, Soluble solids. Ascorbic acid, Acidity, Soluble sugars.

Guava (*Psidium* guajava L.) is a popular fruit, grown successfully throughout tropical and sub-tropical regions of India. Guava is also termed as poor man's apple because it is cheap and easily available to the common man in the plains of Northern and Central India. It is a rich source of certain minerals like calcium, phosphorus and iron which are necessary for human health, it is also rich in vitamin C as reported by Bose and Mitra (1980). Some of the guava cultivars contain vitamin C four to five times more than that present in fresh orange juice. Several palatable food products like jam, jelly, canned fruits, sherbat and non-alcoholic beverages can be prepared from guava fruits. However, sufficient information regarding ratio of surface area : weight, true density and flesh : seed is not available.

The variations of soluble solids, acidity, ascorbic acid and free soluble sugar with maturity of the guava are not available so as to design post-harvest equipments and optimization of the process parameters for its post-harvest handling.

The present experiment was aimed at studying the physical and chemical changes induced in guava during the ripening period. Suitable experiments were conducted to evaluate these changes.

Samples of guava variety ('Lucknow 49'), were collected from the orchard of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. The physical and biochemical parameters were determined at 5 stages of maturity. The change in colour, from green to turning yellow, was considered as a measure of maturity. At each stage of maturity, four replications were taken for analysis. Changes in 6 physical properties namely weight, size, surface area, true density, flesh : seed ratio and moisture content were determined. The samples were also analysed for biochemical properties like total soluble solids, ascorbic acid content, etc. as per the standard procedures given in succeeding paragraphs.

Determination of physical and biochemical properties : The weights of the samples were recorded with the help of triple beam balance (capacity 0 - 3 kg.) The size of guava fruits was determined by taking dimensions from three axis, with the help of Vernier calipers and calculated by using the formula size (mm) = $(a \times b \times c)^{1/3}$ where a, b and c = dimensions of length, width and thickness of guava fruit, respectively. Surface area of the sample was calculated by wrapping the fruit with paper and then encompassing the area of that paper in rectangle and triangle. The sum area represented the surface area of the fruit. Flesh : Seed ratio was calculated by weighing the flesh and its corresponding seed content separately. The flesh weight was divided by seed weight. True density was calculated by toluene displacement method and percentage moisture content was determined by gravimetric method (AOAC 1965). Total soluble solids was determined by a hand refractometer at 200 (range 0-50%) as per AOAC (1970) method. Titrable acidity was determined as percent citric acid by titrating with 0.1 N NaOH using phenolphthalein as indicator and calculated as follows :

$$\% \text{ Titrable acidity (as citric acid)} = \frac{64/1000 \times \text{N. NaOH} \times \text{Titre} \times \text{Volume made up} \times 100}{\text{Volume of sample taken for estimation} \times \text{wt. of sample taken for estimation}}$$

Ascorbic acid content was estimated by titrating the sample with 2-6-dichlorophenol-indophenol and calculated as per the formula given below

$$\text{mg of ascorbic acid/100 g} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract taken for estimation} \times \text{Volume of sample taken for estimation}}$$

The above methods are briefly described by Ranganna (1986). Free soluble sugars were estimated by the procedure of Dubois et al (1956).

Physical properties : Weight of guava/unit size : It is evident from Table 1 that the weight of guava per unit size was increasing during ripening. This may be due to the fact that the moisture content also increased with the advancing stage of ripening. The true density reduced with the advance

*Corresponding Author

TABLE 1. VARIATION OF PHYSICAL PROPERTIES WITH RESPECT TO STAGE OF RIPENING OF GUAVA

Stage	Wt of guava, g	Size, cm	Moisture, %	Surface Area, sq. cm	True density, g/cc	Flesh : seed	Wt. of guava/ unit/size, g/cc	Wt. of guava/ unit area, g/cm^2
100% green	319.25	8.67	82.56	237.14	1.03	2.8 : 1	36.822	1.34
60% yellow	361.75	8.62	83.10	230.00	1.02	2.9 : 1	41.966	1.57
80% yellow	461.00	9.42	83.26	280.51	0.94	3.0 : 1	48.938	1.64
90% yellow	455.25	9.36	85.35	282.50	0.92	3.1 : 1	48.63	1.61
100% yellow	466.75	9.29	85.35	287.78	0.91	3.5 : 1	50.242	1.62

TABLE 2. EFFECT OF THE STAGE OF RIPENING ON BIOCHEMICAL PARAMETERS OF GUAVA

Stage	Total soluble solids, ^{0}Brix	Acidity, %	Ascorbic acid, mg/100g	Free soluble sugar, %
100% green	10.50	0.715	118.53	4.81
60% yellow	11.25	0.677	142.59	5.16
80% yellow	11.50	0.640	155.59	5.73
90% yellow	11.75	0.615	161.40	6.62
100% yellow	12.75	0.555	199.26	7.32
CD 5%	0.620	0.091	29.63	0.443

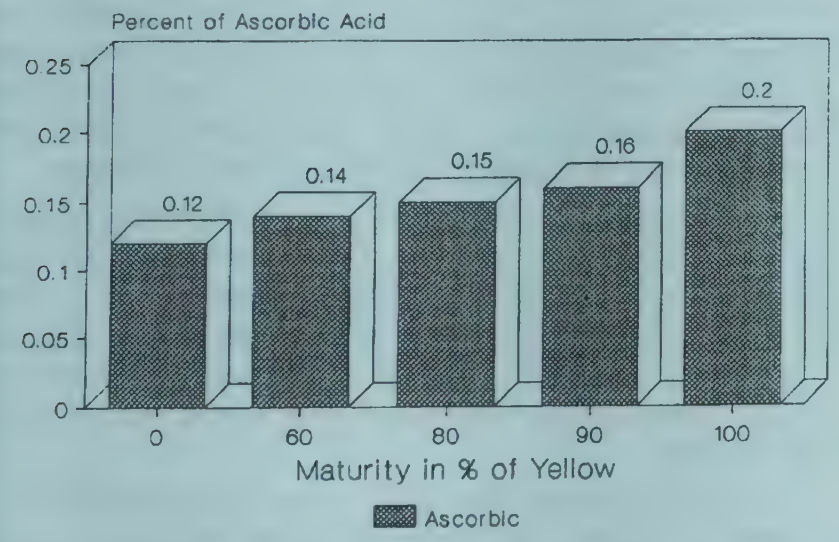

Fig. 1 Variation of ascorbic acid with respect to Increasing yellowness

of ripening stage which may be due to reduction in wt/unit area of guava. Moisture content of fruits of different stages ranged between 82.56 and 85.35% (Table 1). The differences were non-significant. However, it increased with the stage of ripening. Buluk et al. (1995) also reported significant increase in the moisture content during growth and development of guava fruits.

Biochemical parameters : The variation of biochemical parameters with ripening is shown in Table 2. It was observed that the total soluble solids increased with the ripening stage. The highest value of 12.7^0 Brix was observed when the fruits were 100% yellow, whereas the lowest 10.5^0 Brix was observed in 100% green fruit. The difference was significant in 100% yellow fruits, indicating that there existed a correlation between the pigmention and other biochemical changes responsible for increasing the total soluble solids. This finding is in accordance with an earlier work (Gangwar 1972). Titrable acidity decreased with the advancing maturity. Highest acidity (0.72%) was observed in the stage of 100% green fruits. Least percentage (0.56) of acidity was recorded in 100% yellow fruits which gradually decreased with the advancement of repening of fruits, which is in confirmity with finding of Bose and Mitra (1980). The variation of percent ascorbic acid with yellow colour is given in Fig. 1. Significantly higher ascorbic acid (199.25 mg/ 100 g) among all stages of maturity was registered in 100% yellow fruits. Ascorbic acid contents in 90%, 80% and 60% yellow fruits was at par (161.4, 155.86 and 142.59 mg, respectively). Lowest acid content (118.5 mg) among all the stages of maturity was observed in 100% green fruits. Agnihotri and Ram (1972) reported similar observations. Increasing trend with respect to maturity was observed in case of free soluble sugars. Significantly higher percentage (7.33) of free soluble sugars was observed when fruits were 100% yellow. In other stages, the free soluble sugars differed from each other significantly and increased with the ripening of fruits.

References

AOAC (1965) Official Methods of Analysis. 14th edn. Association of Official Analytical Chemists. Washington, DC

AOAC (1970) Official Methods of Analysis, 17th edn. Association of Official Analytical Chemists, Washington; DC

Agnihotri RM, Ram HR (1972) Food Industries J 5(8) : 13-16

Bose TK, Mitra SK (1980) Text Book on Tropical and Sub-Tropical fruits 2nd edn. p 280

Buluk EL, Babiker RE, Tinay AH (1995) Biochemical and Physical changes in fruits of four cultivars during growth and development : Food Chem 54(3) : 279-282

Dubois M, Gilles KA, Hamilton JK, Roebers PA, Smith F (1956) Analyt Chem, 28(3) : 350-356

Gangwar RM (1972) Biochemical Studies on growth and ripening of guava. Indian Food Packer 26(5) : 13-15

Ranganna S (1986) Handbook of Analysis and Quality Control for Fruits and Vegetable Product, 2nd edn. pp 9-10, 105-106

Received 20 October 1999; revised 14 August 2001; accepted 14 December 2001

RESEARCH NOTE

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 96-98

Sugarcane Juice Concentrate - Preparation, Preservation and Storage

PUSHPA SINGH*, H.N. SHAHI, ARCHNA SUMAN AND P.C. JAIN

Indian Institute of Sugarcane Research,
Lucknow - 226 002, India

A process was developed for the preparation of sugarcane juice concentrate with acceptable quality for use as an alternative sweetener. Citric acid alone and in combination with sodium benzoate was used as preservatives, while packing in glass bottles to extend the shelf life of the product under ambient conditions for a period of 8 months. The microbial load was within the permissible limit. The physico-chemical characteristics of the prepared concentrates did not show any significant change except for the slight darkening in colour and increase in reducing sugars.

Keywords : Sugarcane juice, Striking temperature, Concentrate, Citric acid, Sodium benzoate, Microbiological parameters.

The production of sugarcane has an important place in agricultural sector in India. About 40% of sugarcane is used in jaggery and khandsari industries (Baboo et al, 1990) and thus, there is an urgent need to diversify the use of sugarcane to produce value-added products with good keeping quality. Fruit juice concentrates are being relished throughout the world and are prepared by removal of moisture from the fruits under reduced pressure for the retention of aroma and colour. The present work was aimed at preparing sugarcane juice concentrate by dehydrating the fresh cane juice, which is a rich source of sugars (Riffer 1988). Various strategies for its preservation and storage under ambient conditions for use as a ready-to-use sweetener was also explored.

Raw material : Sugarcane juice was extracted by a commercial vertical crusher (10 HP, 7 quintals capacity) from the variety 'CoS 767' obtained from the Experimental Farm of the Indian Institute of Sugarcane Research, Lucknow, India.

Preparation of the concentrate : The cane juice obtained was filtered through a muslin cloth and collected in a storage tank. The juice (10 liters per lot) was poured in an open pan and was heated to facilitate the coagulation of the suspended particles into a gummy colloidal substance. When the temperature reached 85^{0}C which took around 15 - 20 min, the nitrogenous impurities present in the juice started coagulating and started floating on the surface. These were then removed with the help of a long handled strainer.

At this stage, Deola (*Hibiscus esculentus*) extract was added, as it is the most commonly used vegetative product for cane juice clarification (Khanna 1995). This extract was prepared by crushing 2.0 g green stem and mixing it in water (25 ml). The extract was added to the boiling pan in three installments (10:10:5) with an interval of 5 - 10 min.

A golden coloured scum appeared on the surface, which was removed. After complete clarification, the contents were clear, transparent and light brownish yellow in colour. At 99 - 100^{0}C, the juice began to froth, which was prevented by continuous stirring. The juice started to thicken at 105^{0}C. In order to arrive at an optimum colour, aroma and consistency, the concentrates were obtained at various striking temperatures varying from 104-109^{0}C. Based on these trials, it was observed that the best colour and consistency could be obtained at 108^{0}C where the density was found to be 1.56 g/cc. The product resembled natural honey in appearance, clarity and colour. The products, which were obtained at temperature range 105 - 107^{0}C were freely flowing with densities 1.15 g/cc and turned darker within a short span of time (on storage of just 6 weeks). On the other hand, at 109^{0}C, the sample started to crystallize upon few days of storage.

Preservation and storage : To the cooled concentrate, citric acid (0.5%), citric acid and sodium benzoate (0.5% and 0.05% respectively) were added as preservatives. The concentrate was stored in large settling tanks and in capped sterilized bottles (200 ml). In order to make valid comparisons, one set was packed untreated and stored at ambient temperatures.

Physico-chemical properties : The concentrates were analyzed for sugars, ash, moisture, protein and fibre by the

TABLE 1. KEEPING QUALITY OF SUGARCANE JUICE CONCENTRATES STORED AT ROOM TEMPERATURE

Treat-ment	Storage period, months	Colour	Taste	Spoilage
A	0	Golden brown	Good	Nil
	3	Do	Good	Nil
	6	Slight darkening	Slightly Sour	MG
	8	Darkening	Sour	MG
B	0	Golden brown	Good	Nil
	3	Do	Do	Nil
	6	Do	Do	Nil
	8	Slight darkening	Good	Nil
C	0	Golden brown	Good	Nil
	3	Do	Do	Nil
	6	Do	Do	Nil
	8	Do	Do	Nil

A : Untreated, B : Citric acid (0.5%), C : Citric acid and sodium benzoate (0.5% and 500 ppm respectively)

* Resembles the colour of Honey, MG : Mould growth on the surface

*Correspond Author; Present Address : Division of Plant Physiology and Biochemistry, Indian Institute of Sugarcane Research, Raebareilly Road, Lucknow - 226 002, India.

TABLE 2. COMPOSITION OF SUGARCANE JUICE CONCENTRATE

Parameters	Value
Moisture, %	29.8 ± 1.2
Total ash, %	2.1 ± 0.7
Acid insoluble ash, %	0.1 ± 0.05
Crude fibre, %	Nil
Total carbohydrates, %	52.3 ± 1.9
Reducing sugars, %	29.1 ± 7.8
Proteins, %	2.4 ± 0.6
Calcium, mg/100 g	79.0 ± 2.1
Iron, mg/gm	4.7 ± 1.6
Phosphorus, mg/g	86.0 ± 14.7

* Average of three samples

affecting the quality parameters like moisture, pH, colour, reducing sugars and total carbohydrates of the concentrates included the treatments with preservatives and storage period.

The results on the effects of preservatives and storage period on keeping quality indicated that citric acid (0.5%) could effectively check the spoilage in a period of six months without any change in the taste. However, on prolonged storage mould growth was observed on the surface. In order to avoid the mould growth, sodium benzoate (500 ppm) was added along with citric acid and it was found that the combination could check the spoilage effectively for a period of eight months at room temperature. The concentrate without any preservative turned dark in colour (Table 1).

Chemical composition : The chemical composition of concentrate is given in Table 2. Four main factors which would affect the quality parameter of the concentrate were moisture; pH, reducing sugars and total carbohydrates were taken into consideration. The effects of storage period and preservatives on the above-mentioned parameters were studied (Table 3). It was found that there was no significant change in the moisture content of the concentrates. Both storage time and preservatives had non-significant effect on the moisture content of the concentrate. This is explained due to the fact that as the products were sealed in airtight bottles, the chances of absorption of moisture were remote.

TABLE 3. PHYSICO-CHEMICAL CHANGES IN SUGARCANE JUICE CONCENTRATES DURING STORAGE AT ROOM TEMPERATURE

Treatment	Storage period, months	Moisture, %	pH	Colour	Reducing sugars, %	Total carbohydrates, %
A	0	30.9	4.5	130.3	29.5	51.5
	3	29.9	4.6	135.3	35.2	53.9
	6	29.5	4.7	144.3	32.8	54.4
	8	32.3	4.6	140.3	37.1	53.2
B	0	31.6	4.6	130.3	29.5	53.1
	3	30.1	4.6	131.3	35.5	50.9
	6	31.1	4.6	131.0	29.5	57.0
	8	30.6	4.6	132.0	30.6	58.1
C	0	30.6	4.6	126.0	31.0	54.7
	3	31.1	4.5	133.9	31.3	58.5
	6	30.5	4.5	131.0	29.3	56.5
	8	30.6	4.6	130.2	31.9	55.3
CD at 5%						
Treatment		NS	0.09	1.15	1.73	NS
Storage period		NS	NS	1.79	2.02	3.10
Treatment x storage period		NS	NS	3.10	3.48	5.20

A : Untreated, B : Citric acid (0.5%), C : Citric acid and sodium benzoate (0.5% and 500 ppm respectively)

The pH of the concentrate remained unaffected without preservatives. However it changed from 4.5 to 4.7 in the untreated samples, while the addition of preservatives did effect the pH changes but storage time had non-significant effect on them.

The changes in colour were found to be maximum where the concentrates were not treated. Within a span of 8 months,

standard methods (AOAC 1980). The levels of calcium, iron and phosphorus were determined by the acid solution of the ash by atomic absorption spectrophotometer (Ewing 1975, Page and Muller 1982).

Microbiological assay : The fresh and stored samples were subjected to microbiological assay for total bacteria, yeast, moulds, lactic acid bacteria and acetic acid bacteria (ICMSF 1992).

The sensory quality : The sensory qualities of concentrates were assessed on a 100 point scale allotting 20 points each to appearance, clarity, colour, flavour and taste as such and later, on storage, by a panel of 6 scientific staff familiar with the product during zero and 6 months of storage.

Statistical analysis : The statistical analysis was done by factorial design and critical difference (CD) at 5% level of significance between the means was calculated. The variables

TABLE 4. MICROBIAL PARAMETERS OF CANE JUICE CONCENTRATE AFTER SIX MONTHS OF STORAGE AT ROOM TEMPERATURE

Type of microbe	Microbial count, cfu/g
Total bacteria	3.6×10^3
Yeast	45
Moulds	27
Lactic acid bacteria	2.7×10^2
Acetic acid bacteria	0.9×10^2

Average of three samples

TABLE 5. SENSORY EVALUATION OF SUGARCANE JUICE CONCENTRATE DURING STORAGE

Character	Time	Sugarcane juice concentrate			CD at 5%
		A	B	C	
Appearance	0	13.0	14.5	18.3	1.2
	6	9.0	14.9	18.3	0.8
Clarity	0	13.0	14.5	18.5	1.3
	6	9.5	15.3	18.3	NS
Colour	0	13.0	14.5	19.0	1.0
	6	9.0	14.1	18.1	NS
Flavor	0	13.0	14.2	18.4	1.2
	6	9.0	15.5	19.0	1.3
Taste	0	13.0	14.6	18.5	1.5
	6	9.0	13.9	18.9	1.5

NS : Non Significant CD : Critical difference

A : Untreated, B : Citric acid (0.5%), C : Citric acid and Sodium benzoate (0.5% and 500 ppm - respectively)

the colour intensity changed from 130.3 units to 140.3 units, while in the products treated with citric acid, it changed from 130 to 132.0 units. These changes were still lesser in the concentrate treated with citric acid and sodium benzoate. Both the storage time, preservative and their interactions significantly affected the colour intensity (Table 2).

The reducing sugars content in the concentrate is approximately 29.5. It was observed that the storage time, preservative and their interactions significantly affected the reducing sugar. It varied from 29.5% to 37.1% (an increase by 8%) in concentrates not treated with preservatives. This change was found to have reduced significantly in concentrates treated with citric acid (29.5% to 30.6%). The change was almost checked in concentrates treated with citric acid and sodium benzoate. Similar trend was observed in total carbohydrates. It was found that with storage time, total carbohydrates increased in concentrates, which were not treated with preservatives, while the changes were non-significant where the preservatives were added.

The changes in reducing sugars and total carbohydrates are accounted for on the basis of acid catalyzed sucrose hydrolysis mechanism (Jones and Smith 1999). This involves the protonation of glycosic oxygen followed by glycosic bond to form monosaccharides in the form of carboxinium ion. Fructose carboxinium (Fru^+) reacts with water to form two fructose units and another fructose to form difructose dianhydride or possibly even the polyhydroxy phenolic (PPCC) coloured compounds. These changes account for the gradual rise in the colour, reducing and total sugars. However these changes were within the limits, showing no perceptible signs of rancidity even after prolonged storage of 12 months.

The results summarized in Table 4 indicate that the microbiological load in all the samples were well within the permissible limits. Thus the concentrate was safe for consumption even at the end of 12 months of storage under ambient conditions. At zero time, the concentrate with citric acid (0.5%) and sodium benzoate (500 ppm) displayed the best appearance clarity, colour, flavour and taste (Table 5). The untreated concentrate was found to be disliked after 6 months of storage as all the characters underwent drastic changes. The change in colour intensity was measured in terms of optical density using Klett summerson colorimeter (Khanna 1951, Table 5). The optical density showed maximum darkening was in case of untreated concentrates, while the minimum was in concentrate with mixture of citric acid and sodium benzoate.

These results have indicated that addition of mixture of citric acid and sodium benzoate in sugarcane juice concentrate improved the overall acceptability because of its better preservation during storage. The results would thus help standardize the process for preparation and storage of sugarcane juice concentrate, which hold immense potential for its commercialization to produce an item of convenience, which directly fits into the dietary habits.

References

AOAC (1980) Official Methods of Analysis, 13th edn, Association of Official Analytical Chemists, Washington, DC

Baboo B, Solomon S, Srivastava SC (1990) Augmenting jaggery products to meet sugar shortage in India : problems and prospects National seminar on Agrotech and bioenergy for sustained crop and agro productivity, VSP, Pune

Ewing GW. (1975) Instrumental methods of chemical analysis, 4th edn. McGraw-Hill Publication, New York

ICMSF (1992) Compendium of methods for microbiological examination of foods, Vandeszant C, Splittsloesser DF (eds), pp 961-974

Jones JKN, Smith F (1999) Advances in Carbohydrate Chemistry, Academic Press, New York, Vol. 4, p 253

Khanna KL, Chakravorti AS (1955) Juice clarification in jaggery manufacture, Scientific Monograph, pp 33-41

Page AL, Muller RH (1982) Methods of Chemical and microbiological analysis (Buxton DR, Gates KE, eds) Madison, Wisconsin, USA, p 427

Riffer R (1988) The nature of colorants of sugar and cane sugar manufacture in chemistry and processing of sugarbeet and sugarcane. Clarks MA, Godshall MA, (eds) Elsevier Science Publishing Co. Amsterdam, The Netherlands, pp 186-207

Received 4 October 2000; revised 23 April 2001; accepted 14 December 2001

J. Food Sci. Technol., 2002, Vol. 39, No. 1, 99-100

COMPUTATIONAL BIOCHEMISTRY AND BIOPHYSICS : Edited by Oren M. Becker, Alexander D. Mackerell Jr., Benoit Roux, Masakatsu Watanabe. Publisher Marcel Dekker, Inc., New York, Basel. pp 511, Price not stated.

This is an excellent book and can be used as a reference book on theoretical and computational methods on biomolecular research. The editors of this book are renowned scientists and have numerous publications in reputed journals. This book describes living systems in terms of Chemistry and Physics. The book is divided into 4 parts. In the first part on Computational methods, there are 11 chapters on very important topics like Atomistic models and Force fields, Molecular dynamics simulation, Conformational characteristics of flexible biomolecules, Normal mode analysis of biological molecules, Free energy calculations using Molecular dynamics and Monte Carlo simulations, Reaction rates and Transition pathways and Computer simulation of Biochemical reactions with the hybrid Quantum Mechanical (QM) and Molecular Mechanical (MM) methods.

The second part of the book is on Experimental data analysis and it consists of two chapters on X-ray and Neutron scattering as Probes of the dynamics of biological molecules and Applications of molecular modelling in NMR structure determination.

Part 3 is on Modelling and Design and comprises three chapters on Comparative or Homology protein structure Modelling, Bayesian statistics in Molecular and structural biology and Computer Aided Drug Design (CADD).

Part 4 deals with some advanced applications. This section has 5 chapters on Computational approaches to protein folding, Simulations of electron transfer proteins, the RISM-SCF / MCSCF approach for Chemical processes in Solutions, Nucleic acid simulations and Membrane simulations.

In Part I, the chapters are well sequenced so that the reader can easily follow one topic to the next and get familiar with the theoretical methods in biomolecular systems.

The remaining part of the book is so designed that the chapters under each section can be read independently.

Computational methods stated in the book allow information to be extended beyond that obtainable by experimental techniques, thereby facilitating investigations in biological sciences.

At the end of each chapter a number of references have been cited. The references are quite upto date (the latest being year 2000).

A very useful feature of this book is that it to gives a 5-page list of important websites of Internet Resources on some relevant topics keep the reader informed of the latest advances. In addition, most websites listed contain links to other websites. This undoubtedly makes the book a vast source of information.

This book is a useful tool for both theoreticians and experimentalists in all fields of Biophysical and Biochemical research. Consulting the book would benefit both nonspecialists and advanced researchers. However, to be used as a text book, some chapters on preliminary topics could have been included.

Nonetheless, this book is a very good document of the progress made over the last decade in the area of computational biochemistry and biophysics and it is definitely worth buying.

RUNU CHAKRABORTY

DEPARTMENT OF FOOD TECHNOLOGY AND BIOCHEMICAL ENGINEERING, JADAVPUR UNIVERSITY, KOLKATA - 700 032.

ENCYCLOPEDIA OF FOOD MYCOTOXINS : Author Martin Weidenborner : Published by Springer-Verlag, D-69124, Hidelberg, Germany pp 295, 2001, Price : US$99/-

The encyclopedia of food mycotoxins is a rare compilation containing 96 figures and 9 tables. The encyclopedia provides a) a comprehensive list of all foods, which have been contaminated with mycotoxin; b) information on specific mycotoxin and c) relevant molds species responsible for mycotoxin production.

The author has taken great care on listing all foods, which have been reported to be contaminated with mycotoxin. While presenting data on maize, general information on dominant fungi, food processing, fate of mycotoxin and other information is dealt in the beginning (p 127), followed by data on mycotoxin that follow a definite pattern (incidence, degree of contamination, range, country of origin/detection). This information is exhaustively listed (for maize p 128 - 142). Special information is also included concerning contamination of food, for example 6/12 mean six positive samples from a total of twelve and the mean represents the mean of positively contaminated samples. The limit of detection is presented as < x, the values above this level are included in calculating the mean of all positive samples. The author has also listed mycotoxin contaminated foods under *natural occurrence* under specific mycotoxin. If we look up for example aflatoxins (p 11 - 16) general information follows a pattern and under *natural occurrence,* alphabetical listing of contaminated foods is given. But each food is separated by an arrow. The significance of arrow is however, not known. The use of arrow seems to be irrelevant as it does not imply any specific information. This also could be misleading since a beginner seeking information on mycotoxin may think that the first food product is more susceptible than the next one.

While presenting information on specific mycotoxin, a set pattern is followed. The data on Fumonsin B1 (p 98-99) for example are presented, beginning with structural formula, isolation data followed by headings like chemical data, fungal source, natural occurrence, toxicity, detection, possible mycotoxicosis, and *further comments,* which include information

on stability/reduction with words of food processing that are in the middle of the running sentences like baking, drying, cooking in bold letters. The data, in general, on Fumanisins mycotoxins (p 100-104) start with a paragraph on structures and then the specific headings start with fungal source, natural occurrence, toxicity detection and *further comments*. The section *further comments* includes again stability / reduction with food processing words that occur in middle of the sentences like fermentation, canning, nixtamialization milling and steeping in bold. Regulation data for Fumanisins are given under separate head.

The information under the head *further comments* is not consistent and differs with respect to different mycotoxins. In case of ochratoxin data on production, cleaning/milling, malting/ brewing control and regulation aspects of the toxin are presented. For aflatoxin and mycotoxin, the same section has the data on production, detoxification (physical and chemical methods), biotransformation and control. This section does not deal with the regulation aspect of aflatoxin.

Regulation of mycotoxin is an important part and many of the mycotoxin do not have this aspect covered in detail. Regulation is not covered for mycotoxin like zearalenone, deoxynivavalenol, HT-2 toxin, T-2 toxin, aflatoxins B_1, B_2, G_1 G_2 M_1 M_2, chetomin, diacetoxyscirpenol and phomopsin. The author in the preface of the book doesn't brief on the general pattern followed to present data on mycotoxins. The reader is at a loss, while browsing the book. With much effort, one could understand the set pattern of presentation. The appendix (p 271) has a table on mycotoxin legislation, which is very exhaustive.

The encyclopedia also contains compilation of information on mold species. As admitted by the author (see preface) sometimes contradictory information about mycotoxin spectrum of mold species can be found in the literature. Therefore, only *safe* and food relevant mycotoxins of a species and not all known toxic metabolites have been listed. A word like pleosporales leads the reader to a common term fungi, the kingdom of Eumycota via Dothidales and Ascomycota.

One would notice a common term that he/she would not expect, in the special encyclopedia of food mycotoxins is given special status. Words like oxygen, carbon dioxide, cow anorexia, angina, anemia etc., are listed. For example water activity = a_w, it is stated that the growth and mycotoxin production in different substrates are only comparable in terms of their a_w and not their water contents. This statement is too general to have appeared in the encyclopedia.

The title of the book is misleading to the reader. The encyclopedia lists not only food mycotoxins but also molds and others in general terms. The terms food mycotoxins and molds should not have been discussed separately. An artificial divide confuses the reader. They are not various branches of knowledge with reference to mycotoxin. It would have been more user friendly if the food mycotoxins were listed in alphabetical order and other relevant information treated as sub-headings under it. For mycologists and mycotoxicologists the book will not confuse. However, for laymen, the book will mislead. The book also provides no facility of reference to lead the reader to attain further knowledge. List of Tables could have been provided after abbreviation. However, a list of recommended journals is given after literature.

Efforts that have been taken to compile the encyclopedia which is the first of its kind, needs to be recognised. A resource publication an a specialized topic on food mycotoxin is welcome.

E. RATI RAO
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
MYSORE - 570 013

Association of Food Scientists and Technologists (India)
Journal of Food Science and Technology

Instructions to Authors

Journal of Food Science and Technology, devoted to original Research and Development contributions in all branches of Science, Technology and Engineering of Foods and Food Products, is a bimonthly publication of Association of Food Scientists and Technologists (India), Mysore. The journal publishes the following types of papers.

1. Research papers with a maximum length of 16 manuscript pages, including figures, tables and references.
2. Research notes which are limited to a maximum length of 8 manuscript pages, all inclusive.
3. Reviews on specific topics of higher utility and current trends. Any scientist(s) who is expert in his/her field of specialization may send his/her contributions. The reviews should be of about 45-50 manuscript pages. Mini reviews of about 20-25 manuscript pages are also considered.

Submission of Manuscripts

The manuscript submitted to *Journal of Food Science and Technology* will be processed with clear understanding; that the work presented in the paper has not been published before; that is not under consideration for publication elsewhere; that the submission has been approved by all co-authors and by the responsible authorities of the host institute where the work was carried out; and that the manuscript will not be published elsewhere in any form without the consent of the copyright holders.

The manuscript should be submitted in triplicate, typed/printed to double-space on one side of A_4 size/bond paper, leaving 2.5 cm margin on all four sides of the page. The data reported in the manuscript must be original with clear definitions of objectives, materials used, methods employed and without repeating the contents. The authors must strictly adhere to the format of the journal and should consult a recent issue of the journal for style and layout, while preparing their manuscripts. **The manuscript will be returned to authors, if it departs in any way from the required format and style.** Papers essentially of an advertising nature will not be accepted. Foot notes for text are to be avoided. All submissions will be reviewed by two referees and an editorial board member.

Manuscript may be sent enclosing a covering letter providing names, fax numbers, addresses, telephone number and e-mail of three potential referees who would be suitable reviewers for the manuscript by first-class mail to :-

Editor-in-Chief

Journal of Food Science and Technology

AFST(I), CFTRI Campus, Mysore - 570 013 India.

☎ **: +91-821-515557, 518670 Fax : +91-821-518670 e-mail : afsti@sancharnet.in**

Manuscripts

The manuscripts of the research paper should be arranged in the following order.

Title Page

- ❑ The title of the paper is to be typed in capital/lower case letters.
- ❑ Give the authors' names in capital letters, followed by their institutional affiliation.
- ❑ Provide name, address, telephone number and fax number or e-mail details of the individual to whom correspondence and questions should be directed.

Abstract

Each paper should be provided with an abstract of upto **250 words** reporting concisely the objectives, approach and the principal findings of the work and in the form acceptable to abstracting agency. This should be followed by at least **6 keywords** and this should include all the main topics discussed in the paper.

Text

The text should start on a new page with introduction (without heading), Methods and Materials, Results, Discussion, Acknowledgement (if any) and References. Results and Discussion may be combined. Research notes will not have any sub headings except for References.

Materials and Methods must give sufficient details for the work to be repeated. Methods of sampling, number of replications and relevant statistical analysis of data should be indicated. Any hazard must be mentioned and the relevant safety precautions or reference to safety procedures must be made. The use of proprietary names should be avoided. The chemicals are to

be referred by names and not by formula in the text. All vernacular names *eg., pulav, puree, chapatti etc.* must be underlined to indicate those to be in Italic types and briefly described when appearing for the first time. All varietal names such as 'IR 150', 'Pb-56' must be put in single inverted commas. Tables, numbered consecutively with Arabic numbers are to be typed on separate sheet and placed after references section. No vertical lines should be used and the table should not have more than 12 columns. Nil results should be indicated by using ND (not detected), while absence of data by the sign, '-'.

References should be cited at the appropriate points in the text by giving authors' names and year in bracket i.e. (Smith 1990; Smith and Gibbons 1990; Smith et al. 1992) as per Harvard System. A list of references, in alphabetical order, should appear at the end of Results and Discussion section. In case of single author reference, the alphabetical arrangement should be by data, while it should be by co-author when the reference with one co-author is cited. The alphabetical arrangement should be by data in case of the reference with more than one co-authors. Abbreviations such as et al., ibid, idem must be avoided. The titles of all scientific periodicals should be abbreviated as in Chemical Abstracts, Biological Abstracts, Annual BIOSIS, List of Serials and Serial Sources for the BIOSIS Data Base. If the journal is not included in these sources, then the name of the journal is to be given in full. Unpublished data or private communications should not appear in the list, but can be indicated in the text. The examples of the layout of typical references are as under :

Published papers/notes/reviews

Tairu AO, Omotosu RA, Bamiro FO (1991) Studies on oxidative stability of crude and processed yellow nutsedge tuber and almond seed oil. J Food Sci Technol 28 : 8-11

Books/approved methods

a) Hacking AJ (1986) Economic Aspects of Biotechnology, Cambridge University Press, Cambridge.

b) AOAC (1984) Official Methods of Analysis, 14th edn. Association of Official Analytical Chemists, Washington, DC

Chapter in edited books/book series/papers in symposium proceedings/souvenir

a) Kurtzman CP, Phaff HJ, Meyer SA (1983) Nucleic acid relatedness among yeasts. In : Yeast Genetics, Fundamental and Applied Aspects. Spencer JFT, Spencer DM, Smith ARW (eds) Springer-Verlag, New York, pp 139-166

b) Gross E (1975) Subtilin and nisin : The chemistry and biology of peptides and a-b-saturated amino acids. In : Peptides, Chemistry, Structure and Biology : Walter R. Merenhoper J (eds) Proceedings of the Fourth American Peptide Symposium, Ann Arbor, Michigan, USA. pp 31-42

c) Bhalerao SD, Mulmulay GV, Potty VH (1989) Effluent management in food industry. In : Souvenir, National Symposium on Impact of Pollution in and from Food Industries and its Management. Association of Food Scientists and Technologists (India), Mysore. pp 1-31

Reports by specified authors/Institutions

a) Andress EL, Kuhn GD (1983) Critical Review of Home Preservation Literature and Current Research, Co-operative Agreement No. 12-05-300-553. USDA and Pennsylvania State University, Pennsylvania

b) USDA (1977) Home Canning of Fruits and Vegetables, Home and Garden Bulletin 18, United States Department of Agriculture, Washington, DC

Patents

Schmidt GR, Means WJ (1986) Process of preparing algin/calcium gel-structured meat products. US Patent 4 603 054

Thesis

References to Thesis are not acceptable and therefore, should be avoided.

Papers presented at symposia

Stevens KA, Klapes NA, Sheldon BW, Klaenhammer TR (1991) Anti-microbial action of nisin against *Salmonella typhimurium* lip-polysaccharide mutants. Paper presented at 91st American Society for Microbiology, Annual Meeting, Dallas, Texas, USA, 5-9 May

Corresponding author will receive a set of page-proofs for correction of type-setting errors. No other major changes in the paper should be made at this stage.

There are no page charges. 25 off-prints of the paper are provided free of charge to the corresponding author of each published paper.

Copyright

The author(s) should fill and send a signed copyright form while submitting the manuscript, which automatically transfers the copyrights from authors to the journal. Authors' traditional rights will not be jeopardized by assigning copyrights in this manner

Copyright Assignment Form/Undertaking

Journal Name : **Journal of Food Science and Technology**

Manuscript Title _____

_____

Authors _____

_____

I/We hereby confirm the assignment of all copyrights in and to the manuscript named above in all forms and media to the publishers of the journal namely, the Association of Food Scientists and Technologists (India) effective if and when it is accepted for publication by the Editor-in-Chief of the journal. Further, I/We confirm that the manuscript contains no material, the publication of which should violate any copyright or other personal or proprietary right of any person or entity and acknowledge that Editor-in-Chief is relying on this letter in publishing this manuscript, with the undertaking that the present manuscript has not been sent to any other journal for publication. I/We also understand that while assigning the copyright in this manner to Association of Food Scientists and Technologists (India), authors' traditional rights will not be jeopardized.

_____

Date

(Name of the corresponding Author)
with signature)

_____

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

CFTRI CAMPUS, MYSORE - 570 013 KARNATAKA, INDIA

Phone : +91-821-515557, 518670; Fax : +91-821-518670; E-mail : afsti@sancharnet.in

www.afsti.org

PUBLICATIONS OF AFST(I)

PUBLICATIONS	INDIA (in Rs.)		FOREIGN in (US $)	
	Price	Postage	Price	Postage
Symposium on status and prospects of the confectionery Industry in India, 1979.	30	18	12	8
Symposium on by-products from food industries : Utilization and Disposal, 1980	30	12	12	6
2nd ICFOST. 1981 : Sensory analysis, Contaminants and Nutrition	40	18	15	7
Symposium on recent developments in Food Packing 1986	65	18	35	8
Prof. V. Subrahmanyan Commemorative Issue 1980 (part of JFST) contains write-up on various aspects of Food Science and Technology	30	18	12	8
Production and processing of meat and poultry products	100	12	45*	-
IFCON 88 - 2nd International Food Convention and Exhibition : Food Technology Overview	100	18	45*	-
IFCON 88 - 2nd International Food Convention and Exhibition : Abstracts of papers.	100	18	45*	-
Trends in Food Science and Technology : IFCON 88 Proceedings, 1988	300*	-	105*	-
Collective Index (Volume 1-25) (Journal of Food Science and Technology)	200*	-	30*	-
Pollution management in food industries 1989	200*	-	30*	-
Development in milling and baking technology	125*	-	30*	-
Trends in Food Science and Technology : IFCON-93 Proceedings, 1993	1250*	-	200*	-
IFCON-93 Poster Abstracts	125*	-	-	-
IFCON-93 Souvenir	125*	-	-	-
Trends in Food Science and Technology : IFCON-98 Proceedings, 1998				
for invited Speakers & Students	500*	-	50*	-
for others	700*	-	100*	-
IFCON-98 Poster Abstracts	125*	-	-	-
IFCON-98 Souvenir	150*	-	-	-
Modern Trends & Perspectives in Food Packaging for 21st Century ICFOST-2000 Souvenir & Poster Abstracts	200*	-	-	-

(* Including Postage, subject to revision by the postal department)

Payments may be made in the form of DD drawn in favour of **AFST(I)** payable at **Mysore.**

All Enquiries to : **Hon. Exec. Secretary, AFST(I), CFTRI Campus, Mysore - 570 013, India.**

Published by the Secretary AFST (India) CFTRI Campus, Mysore - 570 013 and Printed at
M/s. Skanda Offset Printers, CH-65, 2nd Cross, 7th Main, Saraswathipuram, Mysore - 570 009 Ph. : 540830

Registered with the Registrar of Newspapers for India under No. 24918/64

F-IL
Flavours

Great Flavours.... Great Tastes.....

taste is a valued concept in food....
creating consumer appeal through
taste is our business....

We work intimately with the nature in creating unique innovative functional flavour systems that excite the palate-taste and create new sensations with all commitment and confidentiality to being out the best in food products that goes a long way in market success.

F-IL

FOOD INGREDIENT SPECIALITIES PRIVATE LIMITED

No. 1, Vanagaram - Ambattur Road, Ayanambakkam, Madras - 602 102.
Phones : 0091 - 044 - 635 9536 / 0091 - 044 - 625 0451
Fax : 0091 - 044 - 626 4839 / 0091 - 044 - 625 0452. Telegram : "FOODINGRED"